Cancer Immunology

Bench to Bedside Immunotherapy of Cancers

Nima Rezaei
Editor

Second Edition

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Abbreviations

3'-UTR	3'-untranslated region
3D	Three-dimensional
3-MA	3-Methyladenine
4-OHT	4-Hydroxytamoxifen
5AC	5-Azacytidine
Ab	Antibody
ABC	Adenosine triphosphate-binding cassette
Abs	Antibodies
AC	Adenocarcinoma
ACC	Acinar cell carcinoma
ACC	Adenoid cystic carcinoma
Ad5	Adenovirus serotype 5
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ADP	Anti-adipophilin
Ag	Antigen
AHR	Aryl hydrocarbon receptor
AIA	Ag-induced arthritis
AICD	Activation-induced cell death
AIDS	Acquired immune deficiency syndrome
AIF	Aapoptosis-inducing factor
AILT	Angioimmunoblastic T-cell lymphoma
AIRC	Italian Association for Cancer Research
AIRE	Autoimmune regulator
ALK	Anaplastic large cell lymphoma kinase
ALL	Acute lymphoblastic leukemia
ALP	Alkaline phosphatase
alphaGalCer	Alpha-galactosylceramide
ALPS	Autoimmune lymphoproliferative syndrome
AML	Acute myeloid leukemia
ANCs	Absolute neutrophil counts
ANN	Artificial neural network
ANT	Adenine nucleotide translocase
APC	Antigen-presenting cells
APCP	Adenosine 5'-(α, β-methylene) diphosphate
APCs	Antigen-presenting cells

APECED	Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy
APL	Acute promyelocytic leukemia
APM	Antigen presentation machinery
APS-1	Autoimmune polyendocrine syndrome type I
ARB	Average relative binding
ARDS	Acute respiratory distress syndrome
ASCs	Adult stem cells
ASM	Acid sphingomyelinase
ASPS	Alveolar soft part sarcoma
ATCL	Anaplastic large cell lymphoma
ATLL	Adult T-cell lymphoma/leukemia
ATM	Ataxia telangiectasia mutated
ATO	Arsenic trioxide
ATP	Adenosine triphosphate
ATR	Ataxia telangiectasia/Rad3-related kinase
ATRA	All-trans retinoic acid
B SLL/CLL	B-cell small lymphocytic lymphoma/chronic lymphocytic lymphoma
BAFF	B-cell activating factor
BALs	Bronchoalveolar lavage
BCA	Basal cell adenocarcinoma
BCC	Basal cell carcinoma
BCG	Bacillus Calmette-Guérin
BCR	B-cell antigen receptor
BER	Base excision repair
bFGF	Basic fibroblast growth factor
BLI	Bioluminescence imaging
Bregs	Regulatory B cells
BSO	Buthionine sulfoximine
BTK	Bruton's tyrosine kinase
BTLA	B- and T-lymphocyte attenuator
C/EBPb	CCAT/enhancer-binding protein b
CAFs	Cancer-associated fibroblasts
CaP	Prostate cancer
CARD	Caspase-recruitment domain
CBA	Cytometric bead array
CBR	Clinical benefit response
CC	Choriocarcinoma
CC	Chromophobe carcinoma
CCS	Clear cell sarcoma
CD	Clusters of differentiation
CD40-B	CD40-activated B
CD40L	CD40 ligand
CDC	Complement-dependent cytotoxicity
c-FLIP	Cellular FLICE-inhibitory protein
CFSE	Carboxyfluorescein diacetate succinimidyl ester

CGN	Chromogranin
CHL	Classic Hodgkin lymphoma
CHS	Contact hypersensitivity
CIA	Collagen-induced arthritis
CIC/CRI	Cancer Immunotherapy Consortium of the Cancer Research Institute in the USA
CIHR	Canadian Institutes of Health Research
CIMT	Cancer Immunotherapy
CIP	CIMT Immunoguiding Program
CK	Cytokeratin
CLA	Cutaneous lymphocyte-associated antigen
CLEC9A	C-type lectin domain family 9A
CLL	Chronic lymphocytic leukemia
CLRs	C-type lectin receptors
CMA	Chaperone-mediated autophagy
CMC	Chronic mucocutaneous candidiasis
CML	Chronic myeloid leukemia
CNS	Central nervous system
Con	Concanavalin
CP	Core particle
CpG-A ODN	CpG-A oligodeoxynucleotide
CpG-ODN	CpG oligodeoxynucleotide
CPS	Cancer Prevention Study
CQ	Chloroquine
CR	Complete remission
CRC	Colorectal cancer
CRCC	Clear RCC
CRDs	Cysteine-rich domains
CrmA	Cytokine response modifier A
CRP	C-reactive protein
CRT	Calreticulin
CS	Classic seminoma
CS&T	Cytometer setup and tracking
CSC	Cancer stem cell
CSF-1	Colony-stimulating factor
CSF-1R	CSF-1 receptor
CSF3R	Colony-stimulating factor 3 receptor
CSR	Class switch recombination
c-state	Cytosolic state
CTC	Circulating tumor cells
CTL	Cytotoxic T lymphocyte
CTS	Cathepsins
CTVT	Canine transmissible venereal tumor
CVID	Common variable immunodeficiency
Cyt	Cytochrome
DAMP	Damage-associated molecular pattern
DC	Dendritic cells

DCC	Deleted in colorectal cancer
DC-SIGN	Dendritic cell-specific ICAM-3 grabbing non-integrin
DD	Death domain
DDP	Diamindichloridoplatin
DED	Death effector domain
DES	Desmin
DFTD	Devil facial tumor disease
DHh	Desert hedgehog homolog
DISC	Death-inducing signaling complex
DKO	Double knockout
DLBCL	Diffuse large B-cell lymphoma
DNAM	DNAX-accessory molecule
DNMTs	DNA methyltransferases
DNR	Dominant-negative TGF-ß type II receptor
DNT	Double-negative T
DR	Death receptor
DRMs	Detergent-resistant microdomains
DSB	Double-strand break
DSRCT	Desmoplastic small round cell tumor
DSS	Dextran sulfate sodium
DT	Diphtheria toxin
DTE	Desmoplastic trichoepithelioma
DTH	Delayed-type hypersensitivity
DTR	Diphtheria toxin receptor
DUBs	Deubiquitinases
EAE	Experimental autoimmune encephalomyelitis
EBNA	Epstein-Barr virus nuclear antigen
EBV	Epstein-Barr virus
EC	Embryonal carcinoma
ECL	Electrochemiluminescent
ECM	Extracellular matrix
ECP	Eosinophil cationic protein
EGF	Epidermal growth factor
EGFR	EGF receptor
ELISA	Enzyme-linked immunosorbent assay
EM	Effector memory
EMC	Epithelial-myoepithelial carcinoma
EMSA	Electrophoretic mobility shift assay
EMT	Epithelial–mesenchymal transition
EndoG	Endonuclease G
ER	Endoplasmic reticulum
ER	Estrogen receptor protein
ER+	Estrogen receptor-positive
ERK	Extracellular signal-regulated kinase
ES	Embryonic stem
ES/PNET	Ewing sarcoma/peripheral neuroectodemal tumor
EV	Epidermodysplasia verruciformis

FADD	Fas-associating protein with a death domain
FAK	Focal adhesion kinase
FasL	Fas ligand
FcγRII	Fc receptor II
FDA	Food and Drug Administration
FL	Follicular lymphoma
FLIP	FLICE-inhibitory protein
Flt3L	FMS like tyrosine kinase 3 ligand
Fluc	Firefly luciferase
FRB	FKBP12-rapamycin-binding domain
FSC	Forward scatter light
FZD	Frizzled
GAP	GTPase-activating protein
GBM	Glioblastoma multiforme
GC	Germinal center
GCLP	Good clinical laboratory practice
GEFs	Guanine nucleotide exchange factors
GEM	Genetically engineered mouse
GEMM	Genetically engineered mouse models
GFI1	Growth factor-independent 1
GFP	Green fluorescent protein
GI	Gastrointestinal
GITR	Glucocorticoid-induced tumor necrosis factor receptor-related protein
Gld	Generalized lymphoproliferative disease
Gli	Gli transcription factors
Gln	Glutamine
Glu	Glutamate
GLUD1	Glutamate dehydrogenase 1
GLUL	Glutamate-ammonia ligase
GM-CSF	Granulocyte macrophage colony-stimulating factor
G-MDSC	Granulocytic MDSC
GMP	Good manufacturing practice
GPU	Graphical processing units
GRAFT	Genetically transplantable tumor model systems
GrB	Granzyme B
GSIs	Gamma secretase inhibitors
GSK-3β	Glycogen synthase kinase-3β
GVDH	Graft-versus-host-disease
GWAS	Genome-wide association studies
HAX1	HS-1-associated protein X
HBE	Human bronchial epithelial
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCL	Hairy cell leukemia
HCV	Hepatitis C virus
HD	Healthy donors

HDAC	Histone deacetylase
HDACi	Histone deacetylase inhibitors
HDACs	Histone deacetylases
HEV	High endothelial venules
HGF	Hepatocyte growth factor
HGPIN	High-grade prostate intraepithelial neoplasia
HGS	Human Genome Sciences
Hh	Hedgehog
HIES	Hyper-IgE syndrome
HIF2α	Hypoxia-inducible factor 2-α
HIV	Human immunodeficiency virus
HL	Hodgkin's lymphoma
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
HNC	Head and neck cancer
HP	Human papilloma
HPC	Hematopoietic progenitor cells
HPV	Human papilloma virus
HRG	Histidine-rich glycoprotein
HRP	Horseradish peroxidase
HRR	Homologous recombination repair
HS	Herpes simplex
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem-cell transplantation
HSP	Heat shock proteins
HVEM	Herpesvirus entry mediator
IAP	Inhibitor of apoptosis protein
IB	Immunoblotting
IBCC	Infiltrating basal cell carcinoma
ICAD	Inhibitor of caspase-activated DNase
ICAM	Intercellular adhesion molecule
ICAM-3	Intercellular adhesion molecule 3
ICC	Immunocytochemistry
ICOS	Inducible costimulator
ICOS-L	Inducible costimulator ligand
ICS	Intracellular cytokine staining
IDC	Invasive ductal carcinoma
IDO	Indoleamine 2, 3-dioxygenase
IELs	Intraepithelial lymphocytes
IFN	Interferon
IFNγ	Interferon gamma
IFN-γ	Interferon γ
Ig	Immunoglobulin
IgAD	IgA deficiency
IgE	Immunoglobulin E
IHC	Immunohistochemistry
IHC/ICC	Immunohistochemistry and immunocytochemistry

IHh	Indian hedgehog
IkB	Inhibitor of kB
IKK	IκB kinases
IL	Interleukin
IL-10	Interleukin-10
IL-1Ra	Interleukin-1Ra
IL-1β	Interleukin-1β
IL-2Rα	Interleukin-2 receptor-α
ILC	Invasive lobular carcinoma
IM	Inner mitochondrial membrane
IMPT	Intensity-modulated proton therapy
IMRT	Intensity-modulated radiotherapy
IMS	Intermembrane space
INF	Interferons
iNOS	inducible nitric oxide synthase
IP	Immunoprecipitation
iPS	Induced pluripotent stem
IRF	Transcription factor
ISPC	In silico planning comparative
ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibition motif
ITK	T-cell kinase
IVD	In vitro diagnostic
JAK	Janus kinase
JNK	Jun N-terminal kinase
KARs	Killer activation receptors
KGF	Keratinocyte growth factor
KIRs	Killer cell immunoglobulin-like receptors
KSHV	Kaposi sarcoma-associated herpesvirus
LAT	Linker of activation in T-cell
LC	Luminal cells
LCA	Leukocyte common antigen
LCMV	Lymphocytic choriomeningitis virus
LCs	Langerhans cells
LCT	Leydig cell tumor
LD	Linkage disequilibrium
LIR	LC3 interacting region
LMP-1	Latent membrane protein-1
LNA	Locked nucleic acid
LNs	Lymph nodes
LOH	Loss of heterozygosity
LOX	Lysyl oxidase
LPL	Lymphoplasmacytic lymphoma
Lpr	Lymphoproliferation
LPS	Lipopolysaccharide
LTA	Lymphotoxin-α
LUBAC	Linear ubiquitin chain assembly complex

mAb	Monoclonal antibody
Mac	Macrophages
MAC	Microcystic adnexal carcinoma
MALT	Mucosa-associated lymphoid tissue
MAMP	Microbe-associated molecular pattern
MAPK	Mitogen-activated protein kinase
MC	Molluscum contagiosum
MC	Myoepithelial carcinoma
MCA	Methylcholanthrene
MCC	Merkel cell carcinoma
MCMV	Mouse cytomegalovirus
M-CSF	Macrophage colony-stimulating factor
mDCs	Myeloid-derived dendritic cells
MDS	Myelodysplasia
MDSC	Myeloid-derived suppressor cells
MEC	Mucoepidermoid carcinoma
MEXT	Ministry of Education, Culture, Sports, Science and Technology
MF	Mycosis fungoides
MFI	Mean fluorescence intensity
MGMT	Methylguanine methyltransferase
MGUS	Gammopathy of unknown significance
MHC	Major histocompatibility complex
MIACA	Minimal information on reported results including reporting information on cellular assays
MIAME	Minimal information about microarray experiments
MIATA	Minimal information about T-cell assays
MIBBI	Minimal information on biological and biomedical investigations
MIC-A	MHC class I chain-related A
MIF	Macrophage inhibitory factor
MIG	Monokine induced by interferon-γ
miRNAs	MicroRNAs
MISC	Motility-inducing signaling complex
MKPs	MAP kinase phosphatases
ML-IAP	Melanoma inhibitor of apoptosis protein
MM	Multiple myeloma
M-MDSC	Monocytic MDSC
MMP	Metalloproteases
MMR	Mismatch repair
MnO	Manganese oxide
MOMP	Membrane permeabilization
MPSC	Metastatic pulmonary small cell carcinoma
MSA	Muscle-specific antigen
MSCs	Mesenchymal stem cells
MSF	Migration-stimulating factor
MSI	Microsatellite instability

m-state	Matrix state
mTOR	Mammalian target of rapamycin
MVD	Microvascular density
MYG	Myogenin
MZL	Marginal zone lymphoma
NADPH	Nicotinamide adenine dinucleotide phosphate oxidases
NAIP	Neuronal apoptosis inhibitory protein
NCCD	Nomenclature Committee on Cell Death
NCR	Natural cytotoxicity receptor
ncRNAs	noncoding RNAs
NEC	Neuroendocrine carcinoma
NER	Nucleotide excision repair
NF	Nuclear factor
NFAT	Nuclear factor of activated T cells
NF-κB	Nuclear factor-kappa B
NHANES	National Health and Nutrition Examination Survey
NHEJ	Nonhomologous end-joining
NHL	Non-Hodgkin lymphoma
Ni	Nickel
NiS	Nickel sulfide
NK	Natural killer
NKG2D	Natural killer group two member D
NKT	Natural killer T
NLPHL	Nodular lymphocyte predominant Hodgkin lymphoma
NLRs	The nucleotide-binding oligomerization domain-like receptors
NMC	NUT midline carcinoma
NOD	Nucleotide-binding oligomerization domain
NP	Normal prostate
NPC	Nasopharyngeal carcinoma
NPY	Neuropeptide Y
NSCLC	Non-small cell lung carcinoma
Nt	Nucleotides
NTKs	Neurothekeoma
NUT	Nuclear protein in testis
OARs	Organs at risk
OC	Oncocytoma
ODEs	Ordinary differential equations
ONB	Olfactory neuroblastoma
OPN	Osteopontin
OPRCC	Oncocytic papillary RCC
PAC	Pulmonary adenocarcinoma
PAGE	Polyacrylamide gel, and separated by electrophoresis
PAK	p21-activated kinase
PAMPs	Pathogen-associated molecular patterns
PARP	Poly ADP-ribose polymerase
PAX	Paired box

PB	Peripheral blood
PBMC	Peripheral blood mononuclear cell
PBMCs	Blood mononuclear cells
PC	Prostate adenocarcinoma
PCD	Programmed cell death
PCG	Protein coding gene
PD	Paget disease
PDAC	Pancreatic ductal adenocarcinoma
pDCs	Plasmacytoid dendritic cells
PDGF	Platelet-derived growth factor
PD-L1	Programmed cell death-1 ligand
PE	Phosphatidylethanolamine
PE	Pleural effusion
PEMCs	Pleural effusion mononuclear cells
PET	Positron emission tomography
PFS	Progression-free survival
PH	Pleckstrin homology
PHA	Phytohemagglutinin
PI3K	Phosphatidylinositol 3-kinase
PIDs	Primary immunodeficiencies
PIP3	Phosphatidylinositol-3,4,5-triphosphate
PKB	Protein kinase B
PKC	Protein kinase C
PLAD	Pre-ligand binding assembly domain
PLGC	Polymorphous low-grade adenocarcinoma
PlGF	Placental growth factor
PMA	Phorbol myristate acetate
PMNs	Polymorphonuclear leukocytes
PMT	Photomultiplier tube
PNET/ES	Peripheral neuroectodermal tumor/extraskeletal Ewing sarcoma
PNP	Purine nucleoside phosphorylase
PR	Progesterone receptor
PRC	Polycomb Repressive Complex
PRCC	Papillary RCC
pre-pDCs	Precursor of pDCs
PROTOR	Protein observed with Rictor
PRRs	Pattern recognition receptors
PS	Phosphatidylserine
PSSM	Position-specific scoring matrix
Ptc	Patched dependence receptor
PTCH1	Patched receptor
PTM	Posttranslational modification
PTPC	Permeability transition pore complex
PVDF	Polyvinylidene fluoride
PYGL	Glycogen phosphorylase
QDs	Quantum dots

QoL	Quality of life
RA	Rheumatoid arthritis
RAGE	Receptor for advanced glycation end products
Raptor	Regulatory-associated protein of mTOR
Rb	Retinoblastoma protein
RCC	Renal cell carcinoma
RFK	Riboflavin kinase
RFLPs	Restriction fragment length polymorphisms
RHIM	RIP homotypic interaction motif
RHOH	Ras homolog family member H
RIA	Radioimmunoassay
RICD	Reactivation-induced cell death
Rictor	Rapamycin-insensitive companion of mTOR
RIG-1	Retinoic acid-inducible gene I
RIP	Receptor interacting protein
RISC	RNA-induced silencing complex
RLHs	RIG-I-like helicases
RMS	Rhabdomyosarcoma
ROS	Reactive oxygen species
RS	Reference samples
SA	Sebaceous adenoma
SAP	Signaling associated protein
SBDS	Shwachman–Bodian–Diamond syndrome
SC	Sebaceous carcinoma
SCC	Squamous cell carcinoma
SCCHN	Squamous cell carcinoma of the head and neck
SCF	Stem cell factor
SCID	Severe combined immune-deficient
SCLCL	Small cell lung cancer
SCM	Small cell melanoma
SCN	Severe congenital neutropenia
SCNP	Single-cell network profiling
SCs	Stem cells
SCT	Sertoli cell tumor
SDC	Salivary duct carcinoma
SDS	Shwachman–Diamond syndrome
SDS	Sodium dodecyl sulfate
SEC	Small cell eccrine carcinoma
SED	Subepithelial cell dome
SFB	Segmented filamentous bacteria
Shh	Sonic hedgehog
SHh	Sonic hedgehog homolog
SHM	Somatic hypermutation
siRNA	Small interfering RNA
SIRP-α	Signal-regulatory protein-α
SLAM	Signaling lymphocytic activation molecule
SLE	Systemic lupus erythematosus

SMC	Skeletal muscle cells
SMM	Stabilized matrix method
Smo	Smoothened
SNEC	Small cell neuroendocrine carcinoma
SNP	Single nucleotide polymorphisms
SNUC	Sinonasal undifferentiated carcinoma
SOBP	Spreadout Bragg peak
SOCE	Store-operated Ca^{2+} entry
SOPs	Standard operating procedures
SP	Side population
SP-A	Surfactant protein A
SPECT	Single-photon emission computed tomography
SPIO	Superparamagnetic iron oxide
SPN	Solid pseudopapillary neoplasm
SS	Sjögren syndrome
SS	Spermatocytic seminoma
SSC	Side-scattered light
SSCC	Small cell squamous carcinoma
SSO	Sequence-specific probes
SSP	Sequence-specific primers
SSPCs	Salivary gland stem/progenitor cells
STAT	Signal transducer activator of transcription
STAT1	Signal transducer and activator of transcription-1
STIM	Stromal interaction molecule
SVZ	Subventricular zone
SYN	Synaptophysin
T1D	Type 1 diabetes
T2	Transitional 2 immature
TAA	Tumor-associated antigens
TACI	Transmembrane activator and calcium modulator and cyclophilin ligand interactor
TADC	Tumor-associated dendritic cells
TAM	Tumor-associated macrophages
TAMC	Tumor-associated myeloid cells
TAN	Tumor-associated neutrophils
TAP	Transporter associated with antigen processing
TApDCs	Tumor-associated pDCs
TAPs	Peptide transporters
TAS	Trait-associated SNP
TAs	Tumor antigens
TB	Tuberculosis
TBI	Total body irradiation
tBID	Truncated BID
TC/HRBCL	T-cell/histiocyte-rich B-cell lymphoma
TCF-4	T cell factor
TCL	T-cell lymphoma
TCR	T cell receptor

TDLN	Tumor-draining lymph node
TEM	Tie2-expressing monocytes
TEM	Transmission electron microscopy
TEMRA	Terminally differentiated effector memory
TFBSs	Transcription factor binding sites
TFH	T follicular helper
TGB	Thyroglobulin
TGF-β	Transforming growth factor β
Th	T helper
TIL	Tumor-infiltrating lymphocytes
TIL-Bs	Tumor-infiltrating B cells
TLR	Toll-like receptor
TLT	Tertiary lymphoid tissue
TME	Tumor microenvironment
TNC	Tenascin C
TNF	Tumor necrosis factor
TNF-R	Tumor necrosis factor receptor
TNFα	Tumor necrosis factor alpha
TNF-α	Tumor necrosis factor-α
TNM	Tumor-node-metastasis
TRADD	TNF-receptor-associated death domain
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
Tregs	Regulatory T cells
TSC	Tuberous sclerosis complex
TSGs	Tumor suppressor genes
TSH	Thyroid-stimulating hormone
TSLP	Thymic stromal lymphopoietin
TTP	Time to progression
U1snRNP	U1 small nuclear ribonucleoprotein
UADT	Upper aerodigestive tract
UC	Urothelial carcinoma
UCH	Ubiquitin C-terminal hydrolases
ULBPs	Unique long 16 binding proteins
Unfrac	Unfractionated
UNPC	Undifferentiated nasopharyngeal carcinoma
uPA	Urokinase plasminogen activator
UPP	Ubiquitin-proteasome pathway
UPS	Ubiquitin-proteasome system
USP	Ubiquitin-specific proteases
USPIO	Ultrasmall superparamagnetic iron oxide nanoparticles
UV	Ultraviolet
UVRAG	Ultraviolet radiation resistance-associated gene
VEGF-A	Vascular endothelial growth factor-A
VIM	Vimentin
VINIII	Vulvar intraepithelial neoplasia grade III
VNTR	Variable number tandem repeat
VZ	Varicella zoster

WAS	Wiskott–Aldrich syndrome
WASp	WAS protein
WASP	Wiskott–Aldrich syndrome protein
WGS	Whole genome sequencing
WHIM	Warts, hypogammaglobulinemia, infections, and myelokathexis
WM	Waldenstrom macroglobulinemia
WT	Wild-type
X-IAP	X-linked inhibitor of apoptosis protein
XLA	X-linked agammaglobulinemia
XLN	X-linked neutropenia
XLP	X-linked lymphoproliferative disease
XLT	X-linked thrombocytopenia
YST	Yolk sac tumor

Oncolytic Viruses as Immunotherapeutic Agents

27

Yevhenii Trehub and Andrii Havrilov

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> The most striking sign of leukemia, the excess of leukocytes, disappears, and sometimes the spleen and lymph glands return to their normal size. Yet that the change is not wholly favorable appears from the fact that no case has really recovered… Considering the hopelessness of the ordinary treatment of leukemia, it seems that carefully planned experiments, either with bacterial products or organ extracts, might show a more safe and permanent result.
> —Dock G. (1904) [1].

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27.1 Introduction

Oncolytic viruses are considered as a fundamentally new approach to cancer therapy, which, based on the underlying mechanisms, should be discussed in the context of immunotherapy. Oncolytic viruses (OVs) are viral agents that multiply predominantly

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or exclusively in neoplastic cells and neighboring endothelium, killing them, and do not replicate in cells of normal tissues. Unlike gene therapy, where the virus acts as a gene carrier the product of which is a treatment of a particular disease, the oncolytic virus itself is a means of treatment.

High interest in oncolytic viruses has been observed during the last decade, although the idea of using viruses to fight cancer is not new. Reports of regression of tumors in patients with natural infectious diseases, which now can be retrospectively considered as of viral nature, began to appear since the 1800s [2]. The role of viruses in the treatment of cancer was first mentioned in 1912, when the effect of rabies vaccination on the course of cervical cancer was noted [3]. In 1955, the infection of cervical cancer patients with different adenoidal-pharyngeal-conjunctival virus (APC) serotypes, histological changes in tumor tissue, and the risk of developing a systemic viral disease were investigated deeper and more consciously [4]. In 1949, the effect of viral hepatitis on the course of the Hodgkin lymphoma was investigated, when the volunteer cancer patients were infected with blood plasma or tissue samples of a patient with viral hepatitis. A positive effect was observed in almost half of the cases [5]. In 1952, the infection of patients with various advanced, resistant tumors with the early passage of the West Nile virus (Egypt 101) showed tumor regression in 10% of patients [6]. In 1974 a nonattenuated Mumps virus for the treatment of patients with 18 different types of tumors showed a dramatic effect: a cure or more than 50% regression occurred in 37 of the 90 subjects. At the same time, a killed Mumps virus showed a relatively very weak antitumor effect as a stimulant of immunity in unresponsive melanoma, which indicates the predominant role of the oncolytic but not immunostimulating effect of the virus [7]. These are only a few studies that had been conducted in the field of oncovirotherapy before the 1980s, not to mention the multitude of studies on animals. By the way, Moore in 1949 showed a complete destruction of murine sarcoma 180 on a mouse model under the influence of Russian Far East encephalitis virus under certain conditions [8, 9], which became a milestone in the development of oncovirotherapy. The limiting factor for the widespread use of oncovirotherapy was an inability to restrict the viral process to make it minimally harmful to healthy tissues and limit viral replication to tumor cells alone. Therefore, in the 1970s and 1980s, the research activity around oncolytic viruses was somewhat faded due to certain legal and ethical limitations, but interest in them did not disappear.

At the same time, attempts were being made to reduce the systemic damage of the viruses for the organism. In 1952, Moore notes that the passaging of the virus in a culture of tumor cells increases its tropism 20- to 30-fold to this tumor in vivo [10]. This was the beginning of an era of manipulation of the viruses, although it was still far from real interventions in their structure and genome.

Trying to reduce the harm of viruses a hypotheses of virus competing for the target organ have been put forward: to reduce the harm of a Russian Far East encephalitis virus, it was proposed to simultaneously infect the object with a nonpathogenic neurotropic Newcastle disease virus [11]. This slightly prolonged survival, but the Newcastle disease virus did not show interference with the most oncolytically active at those years Egypt 101 isolate of West Nile virus [2].

Attempts have been made to use viruses that are pathogenic for some animal species to treat tumors of other species. The most successful example was an avian Newcastle Disease Virus. Injected to mice with abdominal cavity carcinoma (Ehrlich ascites carcinoma), it caused a significant tumor response without any manifestations of a viral disease [12]. The very important clue then was the detection of the increase in antitumor immunity after treatment with oncolytic virus—more than 80% of mice cured by the virus did not develop carcinoma after repeated application of this type of cancer cells [13]. This became the basis for understanding that the virus not only causes lysis of the cancer cell but also stimulates anticancer immunity.

However, at that time, the risk associated with an infection of the animal population with a virus that they had never contacted before and had no protection against was underestimated. Such a virus, according to the theory of epidemiology, can adapt, acquire pathogenicity, and increase virulence toward the unprotected species. One of the

viruses used in oncolytic studies was the feline panleukopenia virus, which mutated and acquired the ability to transmit to dogs. It is believed that this virus infected 80% of dogs around the world in the late 1950s as canine parvovirus infection [14, 15].

In the early 1990s, with the advent of DNA recombination technologies and virus-based genetic engineering, oncovirotherapy reached a new stage of development. Now, it has become possible to create recombinant viruses that can only replicate in cells with certain properties—for example, fast-proliferating cells. Martuza's experiment demonstrated the selective activity of the herpes simplex virus with deleted thymidine kinase gene in the malignant glioma tissue [16]. In 1998, the Phase I clinical trial of the G207 virus for patients with brain tumors started in the United States [17], in 2015—the Phase I trial of this virus in children with supratentorial brain tumors [18]. In 2005, H101, a recombinant adenovirus, was approved in China for the treatment of head, neck, and esophageal cancers [19, 20]. In 2015, T-VEC was approved by the FDA for the treatment of melanoma in the United States and in 2016 in Europe and Australia [21–23].

27.2 Model of Oncolytic Virus and Macroorganism Interaction

Immediate realization of the oncolytic potential of the virus occurs, undoubtedly, after its direct interaction with the tumor. This is preceded by the introduction of the virus into the macroorganism—its infection. Depending on the route of administration, which basically can be either intratumoral or systemic, the virus is more or less in contact with the bloodstream, where it is exposed to the primary influence of protective factors that it has to overcome in order to provide the expected effect.

The immune system of the macroorganism was originally considered and indeed is an obstacle to the effective use of oncolytic viruses. Even in the earliest studies in the 1950s, it was observed that active tumor necrosis under the influence of APC virus did not last long due to the eradication of the virus by the host's immune system. In addition, patients who had previously suffered an adenovirus infection showed less response. Viruses which the patient could be contacted with prior to treatment, for example, adenoviruses or poxviruses, are quickly inactivated by the neutralizing antibodies present in the body and demonstrate limited effectiveness. But even in the absence of preimmunization, the viruses rapidly interact with complement and are absorbed by phagocytic cells. Following the injection of vesicular stomatitis virus (VSV) into the systemic circulation, after 2 min, most of the particles become associated with blood cells, and only a small part of them are free in the blood plasma. After 30 min, all the viral particles are already bound to the cells [24]. It turned out that among these cells there are not only ones specialized in virus eliminating but also others which contact with the virus opportunistically. The latter, migrating in the bloodstream, protect the viral particles penetrated into them or adhered on their surface from the immune response and disseminate them into tissues, where the cells migrate to perform their normal functions. Experiments with tumor-antigen-specific T lymphocytes loaded with oncolytic vesicular stomatitis virus and reovirus in vivo showed minimal neutralization of viral particles even at high titers of virus-specific neutralizing antibodies in the animal. In natural conditions, carriers of viruses can be both T lymphocytes and dendritic cells (DCs), which was shown for retrovirus, Newcastle disease virus (NDV), VSV, and reovirus [25–30]. As artificial carriers, different cell lines that can selectively migrate into a tumor or even contact tumor cells are investigated: tumor-antigen-specific T cells, cytokine-induced killer cells, tumor-associated macrophages, mesenchymal stem cells, granulocytes, platelets, and others [31–35]. It is possible to coat the viral particles with polymers, for example, polyethylene glycol or poly-(N-(2-hydroxypropyl) methacrylamide) (pHPMA). This protects the virus from neutralization with antibodies and the T-cell response [36].

In other studies, the best response to OVs in immunosuppressive patients was noted, for example, those with lymphoma or leukemia. Cyclophosphamide was used as an immunosuppressive agent. Many chemotherapeutic agents

are immunosuppressors themselves, so the recent issue is the development of the correct mode of combined chemo-virotherapy, in which the virus would be administrated during a period of slight immunosuppression. In addition, viruses that an individual rarely contacts under normal conditions and against which he does not have neutralizing antibodies (e.g., Seneca Valley virus) still have a theoretical advantage over the common types.

Another obstacle is the permeability of the tumor vessels. The tumor can often have a higher interstitial pressure in comparison with a pressure in the vessels, which makes it difficult to deliver therapeutic agents, including viruses. Chemotherapy, killing tumor cells, somewhat reduces intratumoral interstitial pressure and increases extravasation and intake of substances into the tumor, not affecting directly on vascular permeability [37]. This property should be considered when constructing regimens of combined therapy. Local nitric oxide, bradykinin, nitroglycerin, histamine, local hyperthermia, and low-dose paclitaxel increase vascular permeability and substance leakage into the tumor and enhance oncolytic virus bioavailability [38–40]; systemic angiotensin receptor blockers reduce the collagen deposition inside tumors, which results in the decreasing of intratumoral interstitial pressure [41], VEGF enhances endothelial proliferation and angiogenesis in the tumor, enhancing tumor perfusion by the virus and vascular permeability (see below).

To date, in practical use, only mechanical protection of the virus from immune surveillance and tumor barriers is used so far in a form of direct intratumoral ways of introducing the virus, although this method is sometimes complicated and not always safe for the patient and possible.

27.3 Interaction Between Oncolytic Virus and Tumor

Oncolytic viruses carry with them two mechanisms of antitumor effect: direct cytolysis of tumor cells and enhancement of antitumor immunity. Intracellular replication and accumulation of viral copies in the tumor cell leads to its direct destruction and cell death, resulting in the release of tumor-associated antigens and the provocation of an immune T-cell response [42–45]. In addition, genes of proteins that enhance or modify the immune response and even tumor antigens can be induced into the genome of the virus, which moves the virus to vaccine category.

As stated above, the main task of adapting the virus for use as an oncolytic agent is to make it as affine to tumor cells and associated endothelial cells and minimally pathogenic to normal cells as possible. Some viruses have a natural selectivity in relation to tumor tissue, due to certain features of its altered biology and can be used in a natural, unmodified form. Among such viruses are reovirus, parvovirus, coxsackievirus, and Newcastle disease virus.

The tumor itself with respect to its immunosuppressive microenvironment is an optimal place for the replication of the virus, where it cannot be registered by the immune surveillance in the early stages of the viral process. For example, a number of tumors represent reduced expression of type I IFN and have fewer receptors to it or a disturbed signaling pathway (the pathway that leads to inhibition of cell division and activation of p53). In such conditions, viruses such as VSV, vaccinia, Newcastle disease virus, and mumps virus have an advantage and multiply unhindered [46, 47]. However, the role of type I IFN in the interaction of the tumor with the virus is not completely clear and is probably bivalent, and its formation in the tumor can lead to an increase in tumoristatic or lytic effect (see below).

Knowing the peculiarities and differences of the metabolic or signaling pathways of a cancer cell and the absence of or the altered activity of certain functional proteins in it, it is possible to adapt the virus and make it able to replicate only in conditions of such perverted cell biology. For example, by knocking out viral genes that block the antiviral defense of the host cell, if this defense is absent in the tumor, it is possible to achieve the selective replication of the virus only within the tumor. Among the disturbed metabolic pathways that are potential targets for the virus

selectivity are the defects of the RB/E2F/p16 mechanism, p53, PKR, EGFR, Ras, Wnt, anti-apoptosis, hypoxia conditions, or defects in IFN [48–51]. In general, the mechanism of the virus selectivity can be associated with its penetration into the cell, for example, if the cancer cell expresses unique receptors to which the virus is affine (EGF receptor, Her2-neu, folate receptor, prostate-specific membrane antigen and CD20, and nuclear transcription factors PSA, hTERT, COX-2, and osteocalcin are believed to be potential targets for modified viruses [36, 52]), with a disturbed synthesis of IFN in the tumor (Newcastle disease virus, see below), or with disturbed protective antiviral signaling pathways of the tumor cell (as in T-VEC; see below) [53].

27.3.1 Model of Tumor Destruction Under the Virus Influence

A model of the destruction of tumor formation under the influence of infection with OV is very controversial and, for sure, varies for different tumors and viruses. However, with sufficient confidence, it could be argued that this destruction is multimodal and is mediated by the cooperative impact of several factors. A good model of the complex effect of OV on tumor death is proposed by Mahoney D. on the example of vesiculovirus [54]:

Infection of the tumor cell ultimately leads to its lysis via specific pathways and ultrastructural disorders (immunogenic cell death; see mechanism below) and infection of a number of surrounding tumor cells. At this time, intratumoral resident dendritic cells react to a viral infection (by detecting DAMPs and PAMPs, described below) and activate innate immune response, recruiting NK cells, macrophages, and neutrophils. It is interesting to note that some viruses (in particular, vesiculovirus) can increase the release of type 3 IFN by intratumoral immunocytes, with subsequent increase in the number of NK cell receptors on the tumor cells, making them more vulnerable [55]. Recruited innate immunity cells destroy both infected and noninfected tumor cells. Dendritic cells then absorb tumor and viral antigens, migrate to regional lymph nodes, and present antigens to T lymphocytes, which means activation of an adaptive immune response. Activated antigen-specific T lymphocytes migrate into the tumor and continue destroying its cells. For some viruses, tropism was shown to the endothelium of vessels that supply the tumor (a presumable association with an excess of VEGF). Infection of endothelial cells attracts neutrophils and develops vasculitis and thrombus formation in the vessels of the tumor that leads to ischemic necrosis of the tumor tissue.

27.3.2 Immunogenic Cell Death

Oncolytic viruses, as well as some chemotherapeutic agents and radiotherapy, trigger a specific type of cell destruction. It does not fit completely into any of the classic ways of cell death (necrosis, apoptosis, and autophagy). Until recently, the death of tumor cells due to the effect of any therapeutic agents was considered in the context of nonimmune cell death or arrest of the cell cycle. Immunogenic cell death (ICD) of a tumor cell, or immunogenic apoptosis, is a complex response of a tumor cell to injurious effects, resulting in both apoptosis-like death and activation of a specific immune response to tumor antigens. ICD has been shown for anthracyclines, oxaliplatin, bortezomib, radiotherapy, photodynamic therapy, and viral agents [56–61].

The process of ICD starts when the agent affects certain structures of the cellular matrix and requires a contribution of reactive oxygen species (ROS). ROS cause a stress of the endoplasmic reticulum (ER), but at least, just the presence of ER stress and ROS inside the cell simultaneously is required for ICD initiation. In other words, an ability to induce a ROS-based/ROS-associated ER stress is the determining feature for an ICD inducer. Depending on the way of activation of ER stress, all inducers are divided into two types. Type 1 affects intracellular structures other than ER, triggering its stress indirectly through such targets as cytoplasmic proteins, membrane proteins and channels, and proteins of

Table 27.1 Immunogenic cell death inducers [56, 62–64]

Inducer	Cellular target
Type I inducers	
Anthracyclines	DNA or proteins of DNA replication machinery
Oxaliplatin	DNA synthesis
Bortezomib	ERAD, 26S proteasome, CIP2A
UVC irradiation	DNA
Cyclophosphamide (frequent low-dose administration) [63]	DNA
7A7 (EGFR-specific antibody)	Cell surface receptor (EGFR)
Cardiac glycosides (if combined with chemotherapeutic agents) [62]	Na^+/K^+-ATPase
Vorinostat (HDAC inhibitor)	Nucleus (chromatin structure)
Shikonin	Tumor-specific pyruvate kinase-M2 protein
Wogonin	Mitochondria
Type II inducers	
Hypericin-based photodynamic therapy	Endoplasmic reticulum
Oncolytic viruses	Endoplasmic reticulum

EGFR epidermal growth factor receptor, *UVC* ultraviolet C, *ERAD* endoplasmic-reticulum-associated protein degradation, *HDAC* histone deacetylase, *CIP2A* cancerous inhibitor of protein phosphatase 2A

the DNA replication system. This type mainly includes chemotherapeutic agents and UV radiation. Type 2 agents trigger ER stress impacting directly the ER and disrupt its operation. This type mainly refers to oncolytic viruses [56, 58, 59] (Table 27.1).

ER stress is a state of ER in which it either undergoes synthetic overload and therefore cannot cope with an excessive needs of folding of proteins (physiological stress) or synthesizes pathological proteins that cannot be folded into a tertiary structure properly (pathological stress). Disturbances of protein glycosylation or folding into a soluble form, the presence of mutant proteins, and some viral infections lead to ER stress. Eukaryotic cells have developed a protective mechanism against ER stress—the unfolded protein response (UPR) [65]. UPR is a complex of transmembrane proteins of ER whose domains protrude in both the ER lumen and the cytoplasm of the cell: inositol-requiring protein 1 (IRE1), PKR-like endoplasmic reticulum kinase (PERK), and activating transcription factor (ATF)-6 [66]. These proteins are associated with chaperone glucose-regulated protein 78 (GRP78) in the ER lumen, which detects non-folded or misfolded proteins in ER and releases IRE1, PERK, and ATF-6; they undergo activation by homodimerization and autophosphorylation (but ATF-6 migrates to the Golgi where it is activated by the proteases) [66–68]. Activated PERK inhibits protein synthesis by phosphorylation of eIF-2α (i.e., protein shutoff response); eIF-2α triggers an expression of ATF4 which in turn upregulates expression of CHOP that inhibits a gene encoding anti-apoptotic BCL-2 while enhancing expression of pro-apoptotic BIM. Activated IRE1 triggers an expression of protein degradation enzymes (ERAD). ATF-6 triggers an expression of chaperone genes that refold the misfolded proteins [57]. If an activity of the UPR complex is not sufficient to eliminate ER stress, the described adaptation phase is replaced by an alarm phase and further, through a triggering of signaling pathways such as Fas-associated death domain protein (FADD)/caspase-8-dependent cell death, leads to a cell death [69], which can proceed both via caspase-dependent (apoptosis) and caspase-independent pathway (necrosis) [57] (Fig. 27.1).

Immunogenicity of a cell death is determined by a release of signals into an extracellular environment that indicate a nonphysiological nature of the occurring apoptosis—danger-associated molecular patterns (DAMPs), also called alarmins. DAMPs are intracellular molecules that do not normally come out from the cell but when it is stressed, traumatized, or dying are released into surrounding tissues to be detected by receptors of immune cells. Not all DAMPs are pro-inflammatory—some serve as immunosuppressors to downregulate autoimmune reactions in response to a cell death, thereby providing mechanisms for tolerogenic cell death. Among the latter DAMPs are phosphatidylserine (PS), annexin A1 (ANXA1), death domain 1α (DD1α), and B-cell CLL/lymphoma 2 (BCL2). Main immunogenic DAMPs are adenosine triphosphate (ATP), high-mobility

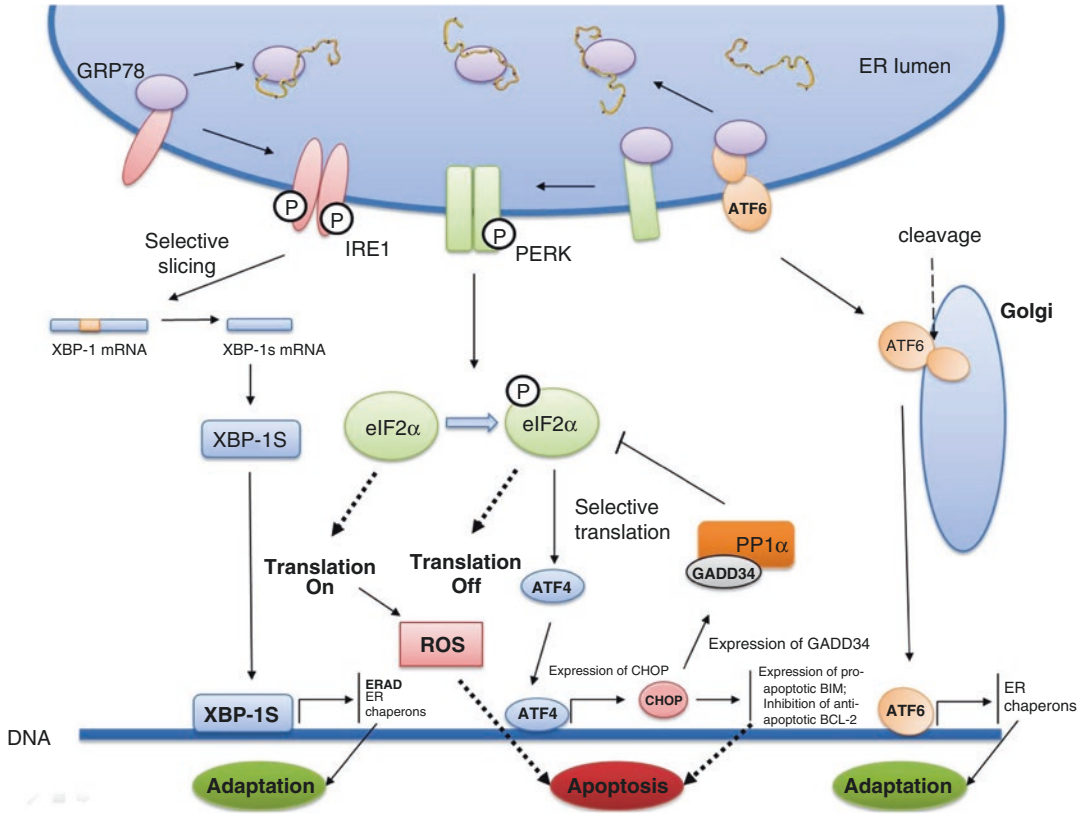

Fig. 27.1 Unfolded protein response. IRE1, PERK, and ATF6 are ER transmembrane proteins that have their domains both in the ER lumen and cytoplasm. GRP78 in normal conditions binds ER luminal parts of IRE1, PERK, and ATF6, attenuating their activity. Accumulation of unfolded or misfolded proteins in the ER lumen leads to GRP78 dissociation and migration into the lumen. Consequently, released IRE1 and PERK are activated through homodimerization and autophosphorylation; ATF6 migrates to Golgi where it undergoes selective proteolysis and subsequent translocation to the nucleus. ATF6 being a transcription factor modulates the expression of genes encoding ER chaperones, which enhance protein folding in ER, and ERAD proteins, which provide degradation of unfolded proteins. Activated IRE1a provides the selective excision of the intron fragment from XBP-1 mRNA (selective splicing). Spliced XBP-1 mRNA translates protein with transcription factor properties that regulates transcription of ERAD pathway proteins and ER chaperons in conjunction with ATF6. Activated PERK phosphorylates eIF2α, which in turn inhibits overall protein translation but enhances translation of ATF4. ATF4 acts as a transcription factor for CHOP, which in turn augments expression of GADD34 and pro-apoptotic BIM but decreases anti-apoptotic BCL-2. GADD34 is a downregulator of the phosphorylated eIF2α activity. Accumulation of ROS due to enhanced protein synthesis along with the expression of pro-apoptotic genes leads to apoptosis [70–73]. *IRE1* inositol-requiring protein 1, *PERK* PKR-like endoplasmic reticulum kinase, *ATF6* activating transcription factor-6, *ATF4* activating transcription factor-4, *GRP78* chaperone glucose–regulated protein 78, *ER* endoplasmic reticulum, *ERAD* ER-associated protein degradation, *XBP-1* X-box binding protein 1, *eIF2α* eukaryotic translation initiation factor 2, *CHOP* C/EBP homologous protein, *GADD34* growth arrest and DNA-damage-inducible 34, *BIM* Bcl-2-like protein 11, *BCL-2* B-cell lymphoma 2 protein, *ROS* reactive oxygen species

group box 1 (HMGB1), heat shock proteins (HSP70, HSP90), and calreticulin (CRT) [59–61]. Their releasing mechanisms, as well as target receptors on immune cells, are presented in Table 27.2.

ER stress, which precedes ICD, is accompanied by an appearance on the surface of the cell membrane of proteins serving as an immunogenic "eat-me" signal for antigen-presenting cells, primarily dendritic cells (DCs). Any ICD, regardless of the inducer, is accompanied by an appearance of calreticulin on the membrane and a release of the immunomodulating molecules such as adenosine triphosphate (ATP) and high-mobility group box

Table 27.2 Main DAMPs occurring in ICD and their brief descriptions

DAMP	Mechanism of release	Immunocytes' receptors	Related mechanisms of cell death
ATP	Actively or passively released	P2Y2 and P2X7	ICD, apoptosis/ secondary necrosis and necrosis
Calreticulin	Mostly surface exposed; sometimes passively released	CD91 (LRP1)	ICD
Heat shock proteins (HSP70, HSP90)	Surface exposure, active secretion, or passive release	CD91 (LRP1), TLR2, TLR4, SREC-1, and FEEL-1	ICD, apoptosis/ secondary necrosis, necrosis
High-mobility group box 1	Mostly passively released; sometimes actively released	TLR2, TLR4, RAGE, and TIM3	ICD, secondary necrosis and necrosis

DAMP danger-associated molecular pattern, *ICD* immunogenic cell death, *ATP* adenosine triphosphate, *LRP1* low-density lipoprotein receptor-related protein 1, *TLR* Toll-like receptor, *SREC-1* scavenger receptor expressed by endothelial cells 1, *FEEL-1* fasciclin EGF-like, laminin-type EGF-like, and link domain-containing scavenger receptor-1, *RAGE* receptor for advanced glycation end products, *TIM3* T-cell immunoglobulin and mucin-domain containing-3

1 (HMGB1) into an extracellular space [60, 74]. Calreticulin (CRT) is an ER-chaperone protein; its migration from the ER to the surface of the cell membrane is a sign of the onset of apoptosis even before its morphological features appear. Translocation of CRT to the surface of the cell membrane is initiated by an activation of caspase-8. The latter leads to an activation of BAX/BAK and cleavage of their substrate Bap31. This is considered necessary for the beginning of migration of CRT [75]. The translocation of CRT is due to its binding to the ERp57 protein, and an CRT/ERp57 complex migrates to the surface [56, 69]. Various proteins of UPR, apoptosis (BAX/BAK/caspase-8), cytosolic Ca2+ play a role in calreticulin transportation to the cell surface. On the membrane, CRT is deposited on low-density lipoprotein receptor-related protein 1 (LRP1) [76, 77]. It is CRT that is considered to be the main signal that causes the immunogenicity of cell death. A blockade of CRT or depletion of CRT with small interfering RNAs (siRNAs) neutralizes the immunogenicity of cell death [76]. Part of CRT is also secreted into an extracellular space, acting as a pro-inflammatory agent and a modulator for DCs: after the impact of CRT, DCs release IL-6, IL-8, and TNF-alpha [78], and the antigen-presentation mechanism is changed—the MHC II pathway is inhibited, and MHC I is activated and, accordingly, a cross-presentation is, with the activation of CD8-T lymphocytes.

HSP90 is another DAMP released during ICD that also migrates to the cell surface and is exposed associated with LRP1. Both surface-exposed CRT and HSP90 interact with specific receptors on the membrane of the immune cell (for example, LRP1 of the DC), which becomes an immunogenic "eat-me" signal for the latter [79–81].

ATP, being a "find-me" signal, binds to P2Y2 receptors of DCs, making them migrate to the apoptosis region. In addition, ATP binds to P2X7 receptors of DCs that activate the NALP3-inflammasome complex, which acts as a trigger for caspase-1 in monocytes [56, 80]. Caspase-1 serves as a protease of pro-IL-1β protein; thus, its activation increases expression of IL-1β by a DC. IL-1β acts as a pro-inflammatory agent; it, together with presentation of tumor antigens, activates the CD8+ T cells and triggers an antitumor adaptive immune response [82, 83].

HMGB 1 is a nuclear protein that is passively released both in necrosis and in the late phase of apoptosis and is an agonist of Toll-like receptor (TLR)-4 of DCs [56]. Its interaction with the receptor stimulates maturation of the DCs and release of pro-inflammatory cytokines. Additionally, HMGB 1 induces multiplication of the IFN-producing Th1 cells clone [84]. The activity of HMGB 1 depends on its redox state. Reduced HMGB 1 behaves as a chemoattractant for leukocytes, disulfide-bond possessing HMGB1—as an inducer of pro-inflammatory cytokines release, and oxidized state is inactive [85]. Moreover, HMGB 1 inhibits immunosup-

pressive Treg cells of the tumor microenvironment [57].

Along with the release of immunogenic DAMPs during ICD, the cell loses tolerogenic "don't eat me" signals. Among such signals is CD47. Moreover, a decrease in the level of CD47 is considered necessary for CRT to manifest its immunogenic properties as an "eat me" signal [58, 86–88].

A picture of ICD caused by a number of OVs is similar to the ICD resulting from other inducers: coxsackievirus B3 [89], measles virus [90], and CD40-ligand expressing adenovirus [91] lead to cell death, which is accompanied by the release of the main described DAMPs—calreticulin, ATP, and HMGB1. However, processes occurring on the ultrastructural level during the OV-mediated ICD is not identical to that caused by other agents. OV takes control of the protein synthesis machinery and mechanisms of cell death, so its course may differ from the described. For example, OV can regulate the cell death apparatus in a way that allows its activation only after all cell energetic resources (ATP) have been depleted [50]. For Newcastle disease virus, it has been shown that it can trigger both caspase-mediated (apoptosis) and caspase-independent (necrosis) death. Also, for this virus, no exposure of HSP70/90 and ATP by the dying cell was observed during ICD. Concerning ATP, this is probably due to its expenditure on viral replication [92].

DCs consume tumor-associated antigens (both endogenous and neoantigens, as well as viral antigens) and present them to the cells of the adaptive immune response in lymph nodes, which in the presence of the immunogenic (but not tolerogenic) DAMPs leads to liberation of pro-inflammatory cytokines (e.g., IL-6/IL-12/IL-1β) [93, 94] by DCs and activation of T cells: polarization of CD4+ lymphocytes into the Th1 and Th17 cells for type-I antibody-dependent antitumor immune reactions (DC-released IFN-γ polarizes CD4+ and also acts as a cytostatic agent for tumor cells) and activation of CD8+ cytotoxic lymphocytes (CTL) by the aid of Th1 cells (cytotoxic lymphocytes cause direct toxic effects on tumor cells mediated through IFN-γ, FasL-CD95 interaction, and perforin-granzyme action) [59, 61, 74, 95–97]. Different OVs presumably can differently activate different components of the adaptive immune response: for example, preferential activation of Th1 was shown for reovirus-mediated oncolysis, while VSV promotes mostly Th17 cells [98]. During the adaptive immune response, a pool of memory T cells is formed, which provide prospective long-term antitumor immunity, mainly maintained by CD8+ T cells.

An obstacle to an effective immune response to the ICD of a tumor cell is the fact that tumor-associated antigens (TAAs) of solid tumors in fact are often self- or close-to-self-antigens. T lymphocytes carrying high-affinity T-cell receptors (TCRs) to these antigens normally undergo negative selection in the thymus and lymph nodes to prevent autoimmunity [99, 100]. Cells with low-affinity TCRs may elude negative selection, but their activity is usually insufficient to trigger a full-fledged immune response due to the immunosuppressive microenvironment in the tumor [101, 102]. ICD decreases the degree of this immunosuppression and increases activity of the low-affinity clone of T lymphocytes for a while, but this pool is quickly suppressed by mechanisms of peripheral tolerogenicity after the fading of ICD, and immunological memory hardly develops. This is especially relevant for chemotherapy regimens, because they have a limited duration of administration due to the development of adverse effects (e.g., severe lymphopenia, which diminishes the antitumor immunity) [99]. From this perspective, OVs seem to be an effective solution as an inductor of ICD—they replicate in a tumor causing ICD for as long, as they still are able to infect other tumor cells; such prolonged ICD stimulates the activity of low-affinity T cells for a long time [59]. But if mutant antigens are present on the tumor, T lymphocytes carrying TCRs to them are not subjected to central (negative selection) and peripheral tolerogenesis, and therefore will be more active in the immune response and memory formation [103].

Another significant potentially positive difference of OVs from other inducers of ICD is that an infected cell, in addition to DAMPs, releases pathogen-associated molecular patterns (PAMPs), which indeed are structural molecules and the products of the vital activity of the virus (like in the infection of normal non-tumorous tissues). Such additional stimulation may enhance the

activity of immunocytes and increase the efficiency of cross-priming of TAAs and, therefore, the immune response to the tumor [57].

Some OVs, in particular Newcastle disease virus, trigger type I IFN response in tumor tissue additionally to ICD [104]. The effect is achieved both by the direct influence of IFN-α and IFN-β on the tumor cell followed by an activation of the antiproliferative effect by p53 induction [46], mediation of the stimulated CD8+ T lymphocytes and macrophages, and release of pro-inflammatory cytokines. The early phase of type I IFN response is the detection of PAMPs by monocytes and DCs via pattern recognition receptors (PRRs). This signal leads to the initiation of IFN-β and then IFN-α expression by these cells. The late phase is the interaction of the released IFN-α and IFN-β with the surface chain of the type I IFN receptor (IFNAR) and start of the synthetic phase of the IFN response, i.e., the signaling pathway resulting in activation of the expression of a wide variety of interferon-stimulated genes (ISGs) that affect the life cycle of the virus at its various stages [105]. It is not yet clear which of the IFN response links are most effective and are of primary importance in the infection of tumor tissue, taking into account the immunosuppressive microenvironment and the disturbed apoptotic and inflammatory signaling pathways of neoplastic cells. IFN response in the tumor may presumably develop after a sufficiently massive infection of the tissue followed by an increase in pro-inflammatory properties of the microenvironment as far as leukocytes infiltration of the tumor occurs (Fig. 27.2). This mechanism requires further study.

27.4 Oncolytic Viruses of Current Interest

27.4.1 Artificially Modified Viruses

Modified oncolytic viruses are mainly normally pathogenic human viruses, which has been induced with specific modifications in their cell invasion or antiviral defense block apparatus, and therefore, they lose their pathogenicity in normal tissues but manifest it in neoplastic cells with defective defense or demonstrate their selectivity to cells with specific membrane receptors. Among the most studied of such viruses are HSV, adenoviruses, and vaccinia, and the most common modifications are blockades of genes attenuating antiviral protection in host cells, changes in proteins responsible for invasion into the cell, and insertions of immunomodulatory protein genes (e.g., GM-CSF) (Table 27.3).

27.4.1.1 Oncolytic Herpesviruses

Talimogene laherparepvec (T-VEC) is the first drug of the OVs group that has proven to be effective in the Phase III clinical trials and is approved for use in Europe [110] and the United States [21, 111, 112].

The virus is constructed on the basis of HSV-1 with mutations in two genes: deletion of *α47* and *γ34.5*, with the insertion of human granulocyte-monocyte colony-stimulating factor (GM-CSF) gene into the locus of *γ34.5* gene [23]. *γ34.5* is responsible for the virus's ability to inactivate the protein synthesis block (protein shutoff) response to the viral invasion of the host cell and thus maintains its replication in the infected cell. Deletion of this gene makes the virus unable to reproduce in a normal cell. But in the neoplastic cell, where the mechanism of the protein shutoff is frequently disrupted, the mutant Δ*γ34.5* virus can still replicate [113]. The *α47* gene serves as an inhibitor of the transporter associated with antigen presentation (TAP) protein. This transporter is involved in the mechanism of antigen presentation and particularly MHC class I expression on the cell surface. Its inhibition makes infected cells invisible for CD8+ CTL [114, 115]. Switching off the *α47* gene enhances expression of Ag/MHC I complexes on tumor cells and antitumor immune response. In addition, inactivation of *α47* enhances expression of a neighboring *US11* gene that additionally increases viral replication in cells [113, 116]. Expression of GM-CSF further enhances maturation of DCs and, consequently, the immune response. In the murine bilateral flank tumor model, a GM-CSF-expressing virus showed an oncolytic effect

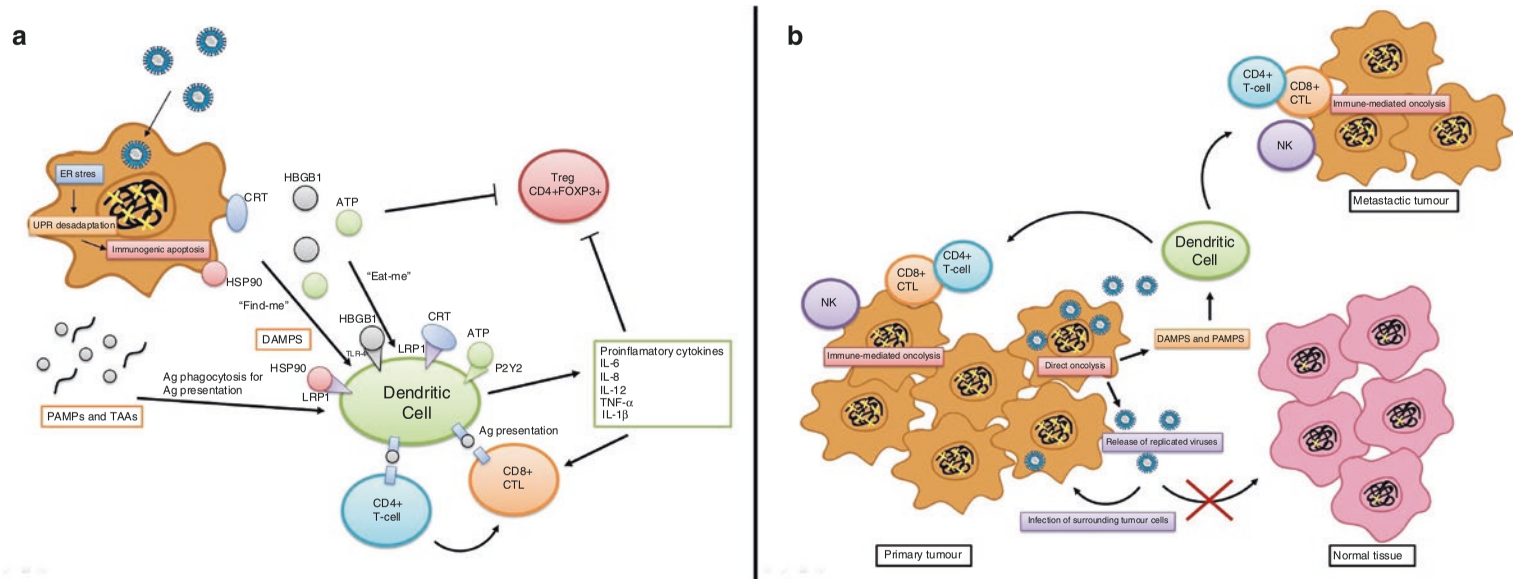

Fig. 27.2 Immunogenic cell death. Intratumoral or systemic injection of OV leads to selective infection of tumor cells due to the dysregulation of their functional pathways (e.g., antiviral defense machinery) or presence of specific receptors or immunosuppressive media (see in the text). Normal tissues are not susceptible to OVs which are either normally nonpathogenic for humans or have genetic modifications providing such selectivity. Infection of cancer cells with an OV causes the consequent response, including ER stress, disadaptation of unfolded protein response pathways, and activation of apoptosis machinery through a caspase-8-dependent cell death pathway. Immunogenic apoptosis is accomplished by the release of DAMPs: surface-exposed CRT and HSP90 which act as "eat me" signals for DCs and extracellular ATP and HMGB1—"find me" signals; TAAs that are processed by DCs for antigen presentation; and PAMPs—viral proteins and nucleic acid that enhance recruitment of immunocytes and are also used for antigen presentation. Replicated viruses released during the cell death invade surrounding cancer cells. Activated DC releases pro-inflammatory cytokines that reduce immunosuppressive properties of tumor microenvironment by Treg attenuation and enhance innate immune response by additional recruiting of NK. Maturated DCs then migrate to regional lymph nodes which present tumor and viral antigens to CTL and Th cells, accordingly initiating adaptive immune response. Tumor (and virus)-specific lymphocytes then infiltrate the primary tumor, as well as distant metastatic tumors that were not exposed to the OV, causing immune-mediated oncolysis [54, 56, 57, 59, 97, 106, 107]. *ER* endoplasmic reticulum, *UPR* unfolded protein response, *DAMPs* danger-associated molecular patterns, *PAMPs* pathogen-associated molecular patterns, *TAAs* tumor-associated antigens, *CRT* calreticulin, *HSP90* heat shock protein 90, *HMGB1* high-mobility group box 1, *ATP* adenosine triphosphate, *LRP1* low-density lipoprotein receptor-related protein 1, *TLR-4* Toll-like receptor-4, *CTL* cytotoxic T lymphocyte, *Th* T-helper, *NK* natural killer, *Treg* regulatory T lymphocyte

Table 27.3 General properties of current OVs under development [108, 109]

Virus family	Virus species	Genome	Mechanism of invasion	Virus strain (name), genetic modification	Current development status
Herpesviridae	HSV-1	dsDNA	Membrane receptors—Glycoprotein D for epithelial cells; HVEM, nectin-1, and nectin-2 for neurons	Talimogene laherparepvec (T-VEC) (Δγ34.5/Δα47/GM-CSF (+))	Approved by FDA for stage IIIB-IVM1a melanoma
Adenoviridae	Adenovirus	dsDNA	Membrane receptors—CAR; HSPG and low-density lipoprotein receptors for hepatocytes	H101 (ΔE1B55K/ΔE3)	Approved by Chinese state Food and Drug Administration for advanced head and neck cancer
				ICOVIR-5 (E1AΔ24/E2F1 (+)/RGD-4C (+) into the fiber knot)	Phase I trial for melanoma
				CG0070 (ΔE3/GM-CSF (+))	Phase II trial for bladder cancer
				OBP-301 (hTERT promoter (+))	Phase I/II trial for hepatocellular carcinoma; phase I for esophageal carcinoma
Reoviridae	Reovirus	dsRNA	Membrane receptors—Sialic acid, JAM-1	Reolysin (non-modified)	Phase III trial for advanced/metastatic head and neck cancer
Paramyxoviridae	NDV	ssRNA	Plasma membrane fusion	NDV (non-modified)	Phase I/II trial for glioblastoma, sarcoma, and neuroblastoma
				NDV oncolysate-pulsed DCs (VOL-DCs) (vaccine)	Received advanced therapeutic medicinal product status
	Measles virus	ssRNA	Membrane receptors—CD46	MV-NIS (sodium/iodine transporter (+))	Phase I/II trial for recurrent ovarian cancer
Picornaviridae	Coxsackievirus	ssRNA	Membrane receptors—CAR, ICAM-1, DAF	Cavatak (non-modified)	Phase I and II trial for melanoma
	Poliovirus	ssRNA	Membrane receptors—CD155	PVS-RIPO (ΔIRES/IRES from human rhinovirus type 2 (+))	Phase I trial for glioblastoma
Poxviridae	Vaccinia	dsDNA	Plasma membrane fusion	JX-594 (ΔTK/GM-CSF (+))	Phase III trial for hepatocellular carcinoma
Rhabdoviridae	VSV	ssRNA	Membrane receptors—LDLR	VSV-hIFNb (IFN-β (+))	Phase I trial for different solid tumors; phase I trial for lymphomas and leukemia
				GL-ONC1 (ΔF14.5L/ΔJ2R/ΔA56R/Renilla luciferase (+)/GFP (+/, β-galactosidase (+))	Phase I/II trial for ovarian, fallopian tube cancer, peritoneal carcinomatosis

Table 27.3 (continued)

Virus family	Virus species	Genome	Mechanism of invasion	Virus strain (name), genetic modification	Current development status
	Maraba virus	ssRNA	Membrane receptors	MG1-MA3 (MageA3 (+))	Phase I/II trial for advanced/metastatic solid tumors
Parvoviridae	Parvovirus	ssDNA	Membrane receptors—Sialic acid, erythrocyte P receptor	ParvOryx (non-modified)	Phase I trial for glioma

OVs oncolytic viruses, *Δ* deletion, *(+)* insertion, *FDA* Food and Drug Administration, *HSV-1* herpes simplex virus-1, *NDV* Newcastle disease virus, *VSV* vesicular stomatitis virus, *HVEM* herpesvirus entry mediator, *CAR* coxsackievirus and adenovirus receptor, *HSPG* heparan sulfate proteoglycan, *JAM-1* junctional adhesion molecule 1, *ICAM-1* intercellular adhesion molecule 1, *DAF* decay-accelerating factor, *LDLR* low-density lipoprotein receptor, *IRES* internal ribosome entry site, *GFP* green fluorescent protein, *MageA3* melanoma-associated antigen 3

both at the site of intratumoral administration and in a distant homologues tumor, whereas the virus without the GM-CSF gene acted only in the primary-injected tumor site [44] (Fig. 27.3). Thus, acomplex theoretical model of the T-VEC virus action can be represented by the following:

At the site of intratumoral injection of the virus, it invades mainly cancerous cells that express an excess of receptors to which the virus has a natural tropism (such as HVEM, nectin-1, and nectin-2) but also normal cells. In normal cells, its replication does not occur since the mechanism of protein synthesis shutoff response is turned on, which cannot be blocked by the virus due to the absence of the *γ34.5* gene. In the tumor cell, the protein shutoff mechanism does not work, so the virus freely replicates in it. During replication, some viral antigens interact with TAP in the Golgi, since the viral protein that normally prevents this event is absent in the virus due to the deletion of *α47*; then, these viral antigens bind with MHC I, and this complex migrates to the cell surface. It promotes virus-specific CD8+ CTL formation, which triggers mechanisms of immune-mediated cell death and attract immunocytes, releasing IFN-gamma. Expression of GM-CSF additionally recruits DCs and macrophages into the tumor and triggers their maturation. Mature antigen-presenting cells then present tumor antigens to CD8+ T cells in lymph nodes; this process stimulates the formation of a tumor-specific clone of CTLs. Lysis of a cancer cell due to the replication of the virus inside it is an achievement of cytoreduction itself. Released from lysed cells, DAMPs, PAMPs and tumor-associated antigens on a background of the immune-activated microenvironment stimulate DCs to trigger an adaptive immune response. Activated antitumor immunity attacks both the primary tumor in which the virus was injected and metastatic foci [110] (see ICD mechanism above).

In Europe, indications for T-VEC is an unresectable melanoma in adults, which is regionally or distantly metastatic (stage IIIB, IIIC, and IVM1a), with no bone, brain, lung, or other visceral diseases [111]. In preclinical studies, T-VEC showed efficacy also in other types of neoplasm, but melanoma was initially chosen for the clinical trial because of the availability of superficial foci for intratumoral virus administration and the known activity of the immune system in this type of cancer.

T-VEC is administrated intratumorally in a maximum dose of 4 ml with a titer of 10^6–10^8 plaque forming units (pfu)/ml diluted in phosphate-buffered saline. The injected dose depends on the size of the tumor: 0.1 ml is used for the tumor smaller than 0.5 cm in the largest dimension; size 0.5–1.5 cm, up to 0.5 ml; 1.5–2.5 cm, up to 1 ml; 2.5–5 cm, up to 2 ml; and lesions more than 4 cm, up to 4 ml. The first injection for the seronegative for HSV-1 patient

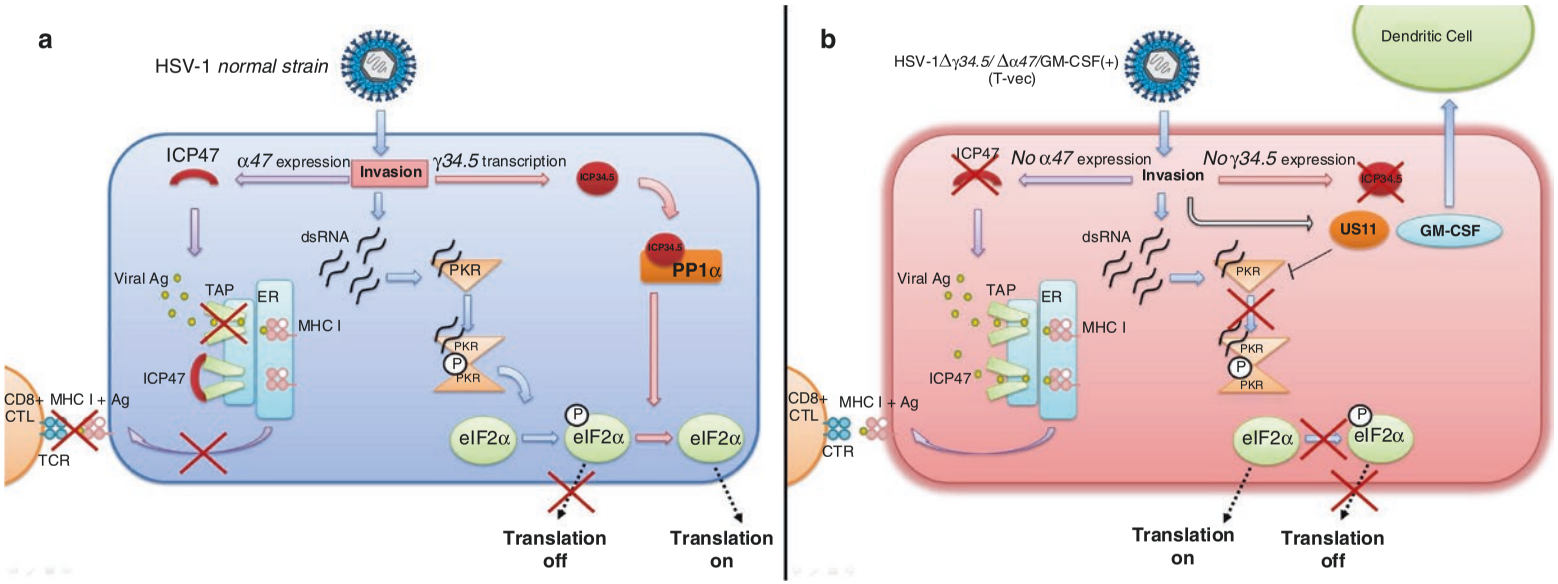

Fig. 27.3 Talimogene laherparepvec (T-VEC) tumor selectivity. (**a**) Following the infection of a normal cell with a wild (normal) HSV-1 virus, a translation of viral proteins starts. Viral dsRNA binds host cell's PKR, being a strong stimulus for its activation. PKR undergoes homodimerization and autophosphorylation. Activated in this way, PKR phosphorylates eIF2α, which causes global protein translation off (protein shutoff response for viral invasion). HSV-1 protein ICP 34.5, a product of *γ34.5* gene, binds PP1α, which redirects its activity in the way of dephosphorylation of eIF2α, accordingly blocking host cell's shutoff response. A product of viral *α47* gene ICP 47 blocks TAP that prevent viral antigen translocation into the Golgi and consequent binding to MHC class I. This inhibits surface exposure of MHC I/Ag complexes and hides the infected cell from CD8+ CTL response. Deletion of these viral genes leads to full-fledged response on viral infection in normal cells. (**b**) Infection of a cancer cell with Δγ34.5/Δα47/GM-CSF (+) modified T-VEC proceeds to the following scenario. PKR in cancer cells is basically attenuated, so it cannot provide eIF2α phosphorylation and protein shutoff. ICP 34.5 does not express in T-VEC (due to *γ34.5* deletion), but it is not necessary in cancer cells as the shutoff response is already blocked. Enhanced expression of the viral *US11* gene (a gene neighboring to *γ34.5*) causes additional inhibition of PKR. The absence of ICP 47 leads to a normal presentation of MHC I/Ag complexes on the cell surface, causing virus-specific CTL-mediated oncolysis in addition to viral oncolysis (ICD). Expression of inserted GM-CSF provides enhanced DCs recruitment to the place of infection and their maturation [113–115, 117–119]. *HSV-1* herpes simplex virus-1, *ICP 47* infected cell protein 47, *ICP 34.5* infected cell protein 34.5, *PKR* protein kinase R, *eIF2α* eukaryotic translation initiation factor 2, *TAP* transporter associated with antigen presentation, *MHC I* major histocompatibility complex class I, *PP1α* protein phosphatase 1α, *CTL* cytotoxic T lymphocyte, *TCR* T-cell receptor, *GM-CSF* granulocyte-macrophage colony-stimulating factor

should be done with a titer of 10^6 pfu/ml solution; the drug is first injected into the largest available tumor and then into others in order of decreasing size until a full one-time dose of 4 ml is applied. The second dose is given after 3 weeks, using a concentration of 10^8 pfu/ml; injections are started with new tumors that have appeared since the previous visit and then the other tumor, starting from the largest, till the full single 4 ml dose is reached. Subsequent visits are conducted at 2-week intervals, with the same regime of injection of the virus. For superficial tumors, the needle is inserted into the central part of the tumor, and the dose is injected into all portions of the tumor, changing the direction of the needle but not removing it, if possible. Each needle removal, as well as injections into different foci, must be accompanied by a needle change. For deeply located formations when it is impossible to insert a needle under visual or palpatory control, ultrasound guidance is recommended. The needle should be removed slowly, during up to 15–30 s, in order to avoid leakage of the drug through the injection site [106, 111, 112].

In the Phase III clinical trial, OPTiM T-VEC showed its efficiency compared with the intratumoral administration of GM-CSF. Durable response rates (which means continuous response of ≥6 months beginning within the first 12 months of therapy), complete responses, and overall survival for patients with IIIB-IVM1a stage melanoma were significantly higher in an arm of talimogene laherparepvec than in GM-CSF. The average overall survival totaled 41.1 months in the T-VEC arm and 21.5 in the GM-CSF one (HR (95% CI) 0.57 (0.40–0.80)). Importantly, not only tumors that had undergone injections responded to the treatment, but also distant tumors did. A total of 64% of injected lesions, 34% of uninjected non-visceral lesions, and 15% of uninjected visceral lesions decreased in size by ≥50% [21]. It means that the theoretical model of the mechanism of action of the virus is confirmed by its practical application.

Adverse effects (AEs) of talimogene laherparepvec are comparatively rare, and it is overall safe for clinical use. Among the most common AEs, pyrexia, chills, flu-like symptoms, general weakness and fatigue, and reactions at the injection site have been noted. Among serious AEs, cellulitis of the injection site with about 2% frequency has been noted. Immune-related AEs such as vasculitis, pneumonitis, and vitiligo have also been noted during talimogene laherparepvec treatment, all being nonserious and occurring in ≤7% of patients [21, 111]. Generalization of infection in the form of herpetic infection is extremely rare and is presented by single cases, and moreover, the study of the genome of the virus-caused generalized infection in those patients revealed it was a wild, but not a genetically modified strain [43].

Although talimogene laherparepvec is generally safe, it is recommended to take certain precautions to prevent the transmission of the virus to a healthy person in close contact. Among these measures, during the whole treatment and 30 days after the last dose, avoid any contact with injection sites and body fluids (use of a condom during sexual intercourse, avoid kissing in the presence of wounds on the oral mucosa in any partner, and use individual dishes and personal care items); for 8 days after each injection, wear water- and airproof dressings at the injection sites, which when utilized should be packed in plastic bags. At the same time, during the treatment, there are no restrictions for patients to visit public places, restaurants, baths, etc. [43].

Contraindications to the use of talimogene laherparepvec are the presence of clinical or laboratory signs of herpetic infection in the patient, current use of antiviral drugs (for example, acyclovir), and severe immunodeficiency (due to HIV, leukemia, lymphoma, immunosuppressive therapy). Patients taking low doses of corticosteroids (up to 10 mg in the equivalent of prednisolone) may be considered as candidates for therapy. The use of the virus in pregnant women and children is not recommended, since this group has not been investigated in clinical trials (although animal studies showed no adverse effect on the fetus) [43].

27.4.1.2 Oncolytic Adenoviruses

As oncolytic agents, serotype 5 adenoviruses are most commonly used. The best-known representatives of oncolytic adenoviruses are H101, which is approved for clinical use in China; ONYX-015, the effectiveness of which is limited; ICOVIR-5; CV706; CG0070; and OBP-301, which are now undergoing clinical trials [120].

The genetic modification of adenoviruses, aimed to increase tumor selectivity, consists in modifying the way of virus penetration into the cell and the process of its replication following the invasion. Adenovirus serotype 5 invasion into the cell occurs in two phases: binding of fiber protein of the virus to the coxsackievirus and adenovirus receptor (CAR) of the target cell [121, 122] and then penetration of the virus mediated by an interaction of arginine-glycine-aspartic acid (RGD) sequence of the penton base and αv integrins on the cell surface [123]. Genetic modification ordering to reduce adenovirus tropism to normal cells (detargeting) consists of deletion in RGD sequence (penton base) gene and induction of the mutation in the AB-loop of the fiber knob [124]. Increased tropism of the virus to tumor cells is achieved by modifying the viral capsid proteins—an insertion of tumor-specific ligands into C-terminus and HI-loop of fiber proteins, L1 loop of the hexon, RGD loop of the penton base, and minor capsid protein IX, which would bind to certain receptors that are present only or predominantly on the surface of the cancer cell [125–128]. The best modification is considered to be those consisting of the insertion of RGD-4C into the fiber knob of adenovirus [129, 130].

A possibility of not only systemic but also local administration of adenovirus is limited by its sequestration during passage through the liver, which is also associated with significant hepatotoxicity. Invasion of the liver cells occurs in a different, CAR-independent way, and therefore, the above-described method of detargeting is not sufficient to minimize the viral tropism to the liver cells [131]. Hepatocytes and Kupffer cells capture viruses by binding their HSPG and low-density lipoprotein receptors to the fiber knob domain but indirectly by the mediation of coagulation factor X and complement component C4-binding protein. Coagulation factor X binds to hypervariable regions (HVRs) of the adenovirus hexon [132, 133]. The genetic modification that prevents this is an induction of a mutation in the coagulation factor X-binding site of the HVR or replacement of the HVR gene with a homologous gene from another adenovirus serotype that does not undergo such sequestration in the liver [120].

Two main methods have been developed in order to limit the replication and cytolytic properties of adenovirus on tumor cells. The first method (or type 1 viruses) is to induce a mutation in the *E1* region. *E1B55K* gene normally functions as an inhibitor of p53 and, consequently, apoptosis of the infected cell. H101 and ONYX-015 viruses carry deletion in this gene, so they can effectively infect and replicate only in tumor cells that lost p53 during progression. *E1A* gene serves to block the Rb-binding domain in Rb/E2F complex of the host cell which results in the release of E2F. The latter in its free state is a transcription factor and activates expression of proteins of DNA synthesis machinery (e.g., DNA polymerase, thymidine kinase, dihydrofolate reductase), which allows the replication of the virus DNA. A mutation of *E1A* gene (*E1AΔ24*) limits replication of the virus only to those cells in which Rb is absent (e.g., malignant glioma or retinoblastoma cells). But this comes with a problem of toxicity: the virus contains an endogenous promoter of *E1A* gene, and therefore, enhanced expression of the defective *E1AΔ24* gene occurs ubiquitously, which becomes toxic (primarily hepato- and hematotoxicity) and creates an obstacle to systemic administration of the virus. To correct this effect, an insertion of the E2F-1 promoter near *E1AΔ24* gene site was performed. This promoter is activated by the free E2F dimer and is blocked by Rb/E2F complex (which is present in normal cells). Activation of the promoter in tumor cells enhances expression of *E1AΔ24*, and its block in normal cells inhibits this expression, which reduces the systemic toxic effects of the virus [120, 134]. The described

modification is present in the last generations of ICOVIR [12, 50].

The second method (type 2 viruses) is that a promoter is inserted into a genome of the virus, which is activated by a specific protein of the tumor cell, which limits the virus replication by a tumor or a specific tissue. This promoter regulates expression of E1A. For example, CV706 virus carries a promoter which is activated by the prostate-specific antigen and therefore multiplies primarily in prostate cancer cells. OBP-301 virus contains a promoter that responds to telomerase reverse transcriptase and, accordingly, multiplies in cells with a high amount of this enzyme [50, 120, 135].

27.4.1.3 H101

H101 virus (Oncorine) has been developed in China and approved by the Chinese State Food and Drug Administration for use as a chemotherapy-combined treatment for advanced stages of head and neck tumors. In the Phase III clinical trial that was conducted in 2000–2004, the virus in combination with chemotherapy showed a 79% positive response rate, compared with 40% for chemotherapy alone [19]. H101 carries a deletion of *E1B55K* (see above) and deletion of the *E3* genes. The latter is responsible for a synthesis of death protein and systemic toxicity of the virus. The mechanism of cell death caused by H101 infection probably lies in ICD, but immunological features and immune response to oncolytic adenoviruses are significantly less studied than that for talimogene laherparepvec. Monotherapy with H101 proves to be not enough effective, presumably because of the difficulties in overcoming barriers formed by the microenvironment of solid tumors by the virus [136, 137]. Therefore, currently, the possibilities of different types of combined therapy are being explored: e.g., a combination of transarterial chemoembolization with simultaneous intraarterial administration of H101 in patients with hepatocellular carcinoma showed 40% 3-year survival rate, while 22% in chemoembolization alone [138]. Histone deacetylase inhibitors in vivo have shown an ability to enhance CAR expression (see above) on the surface of tumor cells (e.g., esophageal squamous cell carcinoma) and, consequently, to increase the H101 infecting activity [137].

Besides H101, H102 and H103 viruses have been developed. H102 carries an alpha-fetoprotein-activated promoter and is therefore able to selectively replicate in hepatocellular carcinoma cells [134]. H103 carries a heat shock protein (HSP) 70 gene, which is a DAMP and enhances immunogenicity of tumor cytolysis. In 2009, the Phase I of H103 clinical trial ended. The results showed an objective response achieved in 11% of patients, and 48% had at least stabilization of the disease [139].

27.4.1.4 The Immune Response to Adenoviruses

The immune response in the context of oncovirotherapy usually consists of two aspects: elimination of the virus due to an activation of antiviral immunity and antitumor response, enhanced by the influence of the virus on the tumor and its microenvironment (i.e., ICD).

Studies with tumor-bearing animals infected with oncolytic adenovirus (VRX-007) have shown that in immunocompetent individuals (both those that were previously immunized with adenovirus and naive), neutralizing antibodies are formed by day 7 after virus administration and at the same time are detected in the tumor tissue; tumor growth stops for 2–3 weeks but then continues, and repeated injections of the virus no longer affect it [140].

On the other hand, the presence of anti-adenoviral immunity plays a role in preventing the dissemination of the virus to normal tissues and provides a certain safety for virotherapy.

Insertion of genes of pro-inflammatory proteins into the genome of adenoviruses in order to strengthen the immunogenicity of infection and cell death is investigated: the abovementioned H103 with an inserted HSP70; proteins GM-CSF, Fas ligand, and IL-27, enhancing maturation and the function of antigen-presenting cells [141]; IL-12, activating T cells [142]; and IFN-α, IFN-β, and IFN-γ, which have a direct antitumor effect

and stimulate the immune response [143–145]. A number of viruses expressing direct-acting antitumor molecules such as TNFα, Fas ligand, and TNF-related apoptosis-inducing ligand (TRAIL) have been developed [146–148]. Most of these options were investigated only in preclinical studies, because due to the success of talimogene laherparepvec, interest in adenoviruses somewhat subsided, but the rapid development of the industry will lead to the need to find the most effective and safe recombinants of viruses, and adenoviruses are the most suitable candidate due to their well-studied genome and great availability for modifications.

27.4.2 Naturally Occurring Oncolytic Viruses

Naturally occuring oncolytic viruses are strains of viruses that are normally not pathogenic to humans, and therefore have minor and easily predicted systemic toxic properties, but exhibit antitumor activity against many neoplasms. They basically do not require any modifications aimed to promote tumor selectivity of the virus, because they do not infect normal human cells, but are able to penetrate and multiply in tumor cells that have lost their mechanisms of antiviral protection. These viruses include Newcastle disease virus, reovirus, parvovirus, and coxsackievirus. A number of natural OVs have modifications that are not associated with an enhancement of their selectivity but with a change in immunogenic properties, for example, VSV with the insertion of IFN-β, tumor antigen libraries and others (Table 27.3).

27.4.2.1 Newcastle Disease Virus

Newcastle disease virus (NDV) is an RNA virus belonging to the *Paramyxoviridae* family. It is basically pathogenic to birds but occasionally can cause an infection in humans in form of conjunctivitis or a mild flu-like syndrome.

NDV is divided into lentogenic (avirulent), mesogenic (medium-virulent), and velogenic (highly virulent) strains depending on the degree of pathogenicity to birds. Such differences are associated with the peculiarities of activation of F (fusion) protein, which provides penetration into the host cell and basically is inactive in its F0 form [149]. F0 is activated by selective cleavage, which in lentogenic NDV is performed only by trypsin-like proteases of the respiratory and digestive tract and, in mesogenic and velogenic by various proteases, for example furin, that is present ubiquitously [123, 149, 150]. This division is important to be understood if talking about viral immunotherapy, since the pathogenicity of NDV is in line with its oncolytic properties. Mesogenic and velogenic NDV can multicyclicly replicate in the human tumor tissue, and they are defined as lytic strains. Lentogenic NDV is prone to be attenuated after the first cycle of replication, and it is a non-lytic strain [151]. Non-lytic strain is interesting mainly in the meaning of being an object for gene-engineering—the artificial modification of the F protein, for example an insertion of the polybasic cleavage site, increases fusogenic and oncolytic properties of the virus and increases the clinical effect in vivo [149, 152–154].

NDV, being an RNA virus, replicate basing on formation of a double-stranded RNA. This structure is a strong inducer of cellular defense mechanisms, consisting in the synthesis of type I (α and β subtypes) and type III IFN, which, by enhancing expression of IFN stimulating genes of innate immunity cells, exhibits antiviral activity in healthy tissues, limiting the spread of the virus. Increased secretion of IFN-α/β at the site of NDV infection has been shown in a number of studies in vitro and in vivo, and generally there is no doubt concerning it. In the tumor tissue, production of IFN and response to it are often disrupted: a weak response of the human fibrosarcoma cell line to IFN-β was shown, due to reduced phosphorylation of IFN-pathway proteins STAT1 and STAT2 and weak activation of IFN-regulated genes [155] and disrupted pathways of apoptosis and antiviral protection (defects of RIG-I, IRF-3, IRF-7), as well as the role of immunosuppressive microenvironment [156, 157]. Reduced production of IFN does not

allow an adequate antiviral response to develop within the tumor at the first stages, allowing the virus to replicate and further infect tumor cells. The defect of apoptosis of infected cells (for example, an excess of anti-apoptotic activity of Bcl-xL [158] and Livin protein [159]) does not allow the virus to be elicited or to limit its replication in the tumor.

Another mechanism that determines the relative insensitivity of normal human cells to NDV is the blockade of viral RNA replication on the basis of a newly produced anti-genome nucleocapsid, which occurs after penetration of the virus into the cell and transcription of its genes. In tumor cells, this stage almost always occurs without the resistance of the host cell.

Cell lines expressing H-Ras and N-ras oncogenes demonstrate greater sensitivity to NDV than their analogs without these oncogenes. Human fibroblasts after N-ras-transfection acquire tumorigenicity and become 1000 times more sensitive to NDV [160]. HaCaT cells are insensitive to NDV before their transformation with H-Ras [161]. All these natural differences form the basis of selectivity of the virus, and NDV replicates 10,000 times faster in human cancer cells than in normal human cells [162].

NDV seems to be an attractive oncolytic agent because its entry into the cell occurs due to binding to sialic acid residues on the membrane that are present on cells of almost all human cancers, which provides a wide range for the use of the virus [163]. In addition, the human population potentially lacks immunity to NDV, so it does not limit its effectiveness (as for adenoviruses). NDV is not inclined to spontaneous recombination and integration into the host's genome. Toxic properties of the virus even in the case of systematic administration are minimal, since it is not basically pathogenic to humans [149].

The mechanism of tumor cell death infected with NDV is similar to ICD induced by other OVs. Among the PAMPs that the NDV-infected cell releases are 5′-triphosphate viral RNA [164], HN protein [165, 166], and double-stranded RNA [161]. These substances react with the pattern recognition receptors (PRR) of innate immunity cells and an early phase of type I IFN response starts as previously described [167, 168].

Among the specificities of ICD caused by NDV is an exposure of hemagglutinin-neuraminidase (HN) and F viral protein to the cell surface. HN protein reacts with Nkp46 PRR of NK cells, which stimulates cytotoxic antitumor properties [165]. HN also activates monocytes and stimulates the release of TNF-related apoptosis-inducing ligand (TRAIL) [169]. HN on the surface of an infected cell enhances an adhesive ability for the better interaction with lymphocytes and is involved in stimulating CD4+ and CD8+ T lymphocytes [170, 171].

In vitro infection of normal and tumor cell lines demonstrated that on the third day after the infection the viability of normal cells ranged 69–95%, while the viability of different malignant cells lines did not exceed 44% [172].

Local intratumoral administration of NDV leads to the tumor infiltration by NK cells and CD8+ and CD4+ FoxP3 lymphocytes, but not by immunosuppressive Treg, and consequently to a significant increase in immunostimulating/immunosuppressive cells ratio. Particles of the virus can be found in a tumor undergone the direct administration of the virus for 96 h following an injection (and possibly further—depending on the method of detection). In a distant metastatic tumor, no virus particles can be detected, but the same lymphocytic infiltration is observed [173]. This indicates the formation of an antitumoral immune response, which confirms the theory of OV-induced ICD.

In preclinical studies, NDV showed its oncolytic effect on many solid tumors, including melanoma, colorectal carcinoma, hepatocellular carcinoma, pancreatic adenocarcinoma, pleural mesothelioma, and glioblastoma. In clinical trials, the virus was used both as a therapeutic agent and for the production of antitumoral vaccines in the form of tumor viral oncolysates (see below): for the treatment of glioblastoma multiforme [174, 175], colorectal carcinoma [176], pancreatic adenocarcinoma [177], breast adenocarcinoma [178], renal carcinoma [179], and others. A 10-year follow-up of patients with stage II malignant mela-

noma who received NDV as adjuvant postoperative therapy showed a 60% survival rate (while observations of such patients receiving standard treatment showed a survival rate of up to 33%) [180].

In 1993, Csatary tested MTH-68/HVVV strain in a placebo-controlled Phase II trial for the treatment of various advanced chemorefractory cancers, where a completely new route of administration of the virus was proposed: inhalations of viral particles at a dose of 4000 U/day, twice per week for 6 months, aimed on targeting pulmonary metastases. The effect was significant—a 2-year survival rate was 21% in the NDV arm and 0% in the control. The treatment was well tolerated, with no significant AEs [181].

In 2002, in Phase I clinical trial of the PV701 strain involving 79 patients with advanced chemoresistant tumors, a spectrum of the adverse effects of the virus was investigated. The most common AE was an influenza-like syndrome, occurring after the first dose but decreasing with subsequent administrations. Dose-limiting effects were dyspnea, diarrhea, and dehydration. Desensitization with minimal initial doses was proposed to address AEs, which increased the maximum tolerated dose tenfold [182, 183]. It is not completely clear how this desensitization affects the effectiveness of therapy, but its effect on toxicity was well-defined. The result of the trial demonstrated a complete response observed in one patient, a partial response in one patient, and minor responses in seven patients. Fourteen patients were progression-free for 4 months to over 30 months.

Non-lytic NDV strain was studied in 14 patients with glioblastoma. One patient had a complete response; all others had progressive disease [175].

To date, the evidence base is not sufficient for a final conclusion on the effectiveness of NDV as an immunotherapeutic drug. The available data clearly indicate that the virus has a potential and requires further research and more extensive clinical trials.

NDV is also studied as an antitumor vaccine in the form of oncolysates or whole-cell vaccines. These vaccines generally have proven to be safe and effective in uncontrolled clinical trials. A clear conclusion about the degree of clinical benefit is not yet available, and it is necessary to conduct controlled trials to make the final conclusion [149].

An interesting approach is proposed by Schirrmacher: a modification of autologous tumor cells taken during resection of the primary focus in a metastatic disease by NDV, to enhance the immunogenic properties and to use these tumor cells as a vaccine. In 2009, the results of the Phase II/III clinical trial of the autologous tumor vaccine modified with non-lytic Newcastle disease virus (ATV-NDV) for postoperative treatment of colorectal cancer with liver metastases were published. In patients with colon cancer, the 9- to 10-year survival rate differed significantly: 21.4% in the control group and 69.2% in the ATV-NDV group. It is interesting that no significant differences were noted in a rectal cancer subgroup [184, 185].

Later, Schirrmacher and others in the Immunological and Oncological Center in Cologne, Germany, modified the ATV-NDV vaccine by adding human DCs. The new vaccine was named viral oncolysate-pulsed DCs (VOL-DCs). This combination increases the efficiency of antigen presentation by cells, as the density of contact of the DCs with tumor antigens increases since the process begins in vitro even before administration to a patient. Exogenous antigen-presenting DCs stimulate maturation of tumor-specific T cells in the patient's body [168]. A proposed complex administration regimen is as follows: the patient receives injection of NDV and hyperthermia up to 38.5–40.5 °C as a pretreatment. After that, the VOL-DC vaccine is administrated [186]. Hyperthermia is a favorable background for enhancing immune responses. NDV triggers oncolysis and ICD of tumor cells that prepare the immune system by stimulation of the formation of a pool of VOL-specific lymphocytes, mostly CD4+ helpers. With the administration of the VOL-DC vaccine against a background of such an activated immunological status, the release of chemokines CCL3 is enhanced at the site of injection. This stimulates active migration of DCs to the regional lymph nodes, and CD4+ helpers increase efficiency of

lymphocyte stimulation by DCs during the antigen presentation, improving the effect of vaccination [187]. VOL-DCs in 2015 received an approval for individual use in cancer patients as an advanced therapeutic medicinal product [168].

Genetically modified strains of NDV are developed and show a good effect. Among the modifications, as mentioned above, are increased fusogenicity by changing the F protein; insertion of NS1 protein (from influenza A virus) that alters immune response by inhibiting the type I IFN response and apoptosis [188]; arming with pro-apoptotic rFMW/AP proteins from chicken infectious anemia virus [189]; cytokines IFNγ, GM-CSF, IL-2, and TNFα [152]; immunoglobulins against ED-B fibronectin [190]; and insertion of tumor-associated antigens genes [191].

27.4.2.2 Reovirus

Reovirus (respiratory orphan enteric virus, genus *Orthoreovirus*, family *Reoviridae*) is a non-enveloped RNA virus that is ubiquitous, affecting the upper respiratory tract and the gastrointestinal tract with minimal clinical manifestations [192]. There are no known serious human diseases associated with reovirus [193]. The asymptomatic course of infection and the ubiquitous prevalence of the virus cause a high frequency of seropositivity to reovirus among the human population [194].

There are three serotypes of mammalian reovirus. Their prototypes were isolated in children with different manifestations of infection or without them. Type 3 Dearing (T3D), isolated from a child with diarrhea, is most widely studied for its oncolytic properties today, although other serotypes also show these properties [195].

The selectivity of T3D reovirus on normal and transformed cells has been studied back in the 1980s, and it was noted that normal cell lines are resistant to infection of the virus, whereas the virus causes cell lysis in transformed cells and the HeLa cell line [196].

Selective oncospecificity of reovirus is associated with the surface receptor of epidermal growth factor (EGFR) and its signaling pathway Ras. The Ras pathway is a proto-oncogene; it is associated with the control of the cell cycle, proliferation, differentiation, and apoptosis of the cell. During transmission of the signal from the EGF membrane receptor, Ras changes from a guanosine diphosphate (GDP)-bound form into an active guanosine triphosphate (GTP)-bound form, triggering the subsequent pathway elements. Mutation of the Ras gene can lead to a stabilization of the active GTP-bound Ras, and the pathway remains active regardless of the presence of EGF stimuli [197], and the cell acquires an ability of uncontrolled proliferation. Such a transformation can occur in another protein of this signaling path—RAF, which leads to the same effect. Hyperactivity of the Ras pathway is often found in cancer cells: up to 30% of all tumors [198], up to 90% of pancreatic cancer, 50% of colorectal, and 40% of lung cancer [199]. Normally, the antiviral protective mechanism of the cell reacts to invasion of reovirus as follows: double-stranded virus RNA (dsRNA) activates protein kinase R (PKR) by binding to the N-terminal domain. Activated PKR inhibits translation of viral proteins, thereby realizing the viral replication blockade (as in T-VEC antiviral response; see Fig. 27.3). Hypothetically, the elements of the Ras pathway system (probably its Ras/RalGEF/p38 part) can inhibit PKR activity [198, 200, 201], and therefore tumor cells with a highly active Ras system are very susceptible to reovirus infection.

However, there is evidence that the mechanism of oncospecificity of the virus is associated with other features of cell biology. In vitro on the squamous cell carcinoma of the head and neck cell lines it was shown that sensitivity of the cells to reovirus did not correlate with a degree of activity of their Ras system, and stimulation or inhibition of EGFR and blockade of MAPK, PI3-K, and p38MAPK elements of the Ras pathway did not affect the cytotoxicity of the virus and the rate of growth of the infected tumor. Inhibition of phosphorylation of PKR (i.e., its artificial inactivation) also did not significantly increase sensitivity of primary resistant cells to reovirus. These data cannot be accepted as the only truth, but it should be remembered that based on this

information not only patients with biomarkers of increased activity of EGFR/Ras/MAPK pathway should be selected for reovirotherapy. Similarly, the criteria for selecting patients for clinical trials should not be a positive EGFR/Ras/MAPK status only [202].

One of the factors of cell's susceptibility to reovirus is the number of specific receptors on the cell surface—junctional adhesion molecule-1 (JAM-1) [203], but there are data that contradict this fact too [202]. The number of co-receptor sialic acid residues on cell membranes may also play role [193].

The mechanism of cell death under the influence of reovirus is thought to be caspase-dependent apoptosis that occurs with a participation of TRAIL and caspase-8 pathways, which was mainly observed for melanoma cells and for several other tumors [204, 205]. Additionally, necroptosis was shown in head and neck squamous cell carcinoma cell lines [206]. An immune response to tumor invasion by the virus and generally cell death occurs according to the common mechanism of ICD: recruitment of DCs, activation of NK and CD8+ T lymphocytes, and formation of antitumor immunity [207].

Due to the high degree of anti-reoviral immunity in the human population and rapid appearance of neutralizing antibodies even at the first contact of a nonimmune individual with the virus, the immune response is a significant limiting factor for systemic intravenous administration of reovirus [193]. The use of reovirus in animal models in combination with immunosuppressive cytotoxic agents such as cyclosporin A, cisplatin, and cyclophosphamide showed a better effect compared to monotherapy, partly because of reduced inactivation of the virus by neutralizing antibodies [208, 209]. Cyclophosphamide, in addition, selectively inhibited Treg activity and antibody formation in response to reovirus and at the same time somewhat modulated the antitumor adaptive response by increasing activity of the T cells. It was also shown that the combination of cyclophosphamide and reovirus with IL-2 can further increase efficiency, probably by enhancing the NK cell response to the tumor [210].

On the other hand, in the experiment with murine tumor models, injection of reovirus to naive mice had minimal effect, while mice immunized against reovirus 2 weeks prior to treatment and having specific antibodies showed a much better tumor response and survival [211]. It supports the significant role of immune response in reoviral oncolysis, and therefore, it is necessary to find a balance between the maximum possible immunosuppression and the minimum necessary immunocompetence for the effective use of OVs in general.

In Phase I clinical trials, a good tolerability and an absence of dose-limiting adverse reactions to reovirus were shown in both intratumoral (in patients with subcutaneous tumors, prostate cancer, and malignant glioma) and intravenous administration (various solid tumors, metastatic colorectal cancer, multiple myeloma), including in combination with chemotherapeutic agents [212–216]. The maximum administrated dose was set on the level of 3×10^{10} TCID(50) (tissue culture infectious dose 50) per injection for 5 days per week, repeated every 4 weeks. However, the maximum tolerated dose wasn't achieved. Among AEs noticed during Reolysin therapy are grade 1 and 2 flu-like symptoms—fever, fatigue, nausea and vomiting, and headache, which didn't depend on dose and cycle—and among grade 3 toxicities—flu-like symptoms and uncomplicated lympho- and neutropenia [217]. Combination of reovirus with chemotherapeutic agents like docetaxel also showed low toxicity: the frequency of grade 3 and 4 toxicities, like neutropenia, was relevant to those for docetaxel monotherapy [212].

A combination of reovirus with carboplatin and paclitaxel in 19 patients with refractory to preceded chemotherapy with platinum-containing agents in advanced head and neck malignancies (mostly squamous cell tumors) has shown an achievement of a complete or partial response in 42% and stabilization in 32%. The median overall survival was 8.9 months that is significantly longer than in other second-line regimens [218]. In a similar study with 13 patients, a partial response was achieved in 31% and at least stabilization during 12 weeks in 46% [219].

The same combination was studied in patients with metastatic non-small cell lung cancer with a mutation in the Ras system. The results are median progression-free survival of 4 months, overall survival of 13.1 month (95% CI: 9.2–21.6), and 1-year survival rate of 57% [220]. Phase II clinical trials were conducted for metastatic small-cell lung cancer; melanoma; ovary, peritoneum, and fallopian tube malignancies; and unresectable pancreatic cancer [221].

Phase III clinical trial of a combination of IV reovirus with carboplatin and paclitaxel in comparison with carboplatin and paclitaxel alone in patients with advanced or metastatic head and neck tumors involving 167 patients is being conducted. Of these, for 118 patients with locoregionally advanced tumors (with and without metastases), results were obtained: median progression-free survival was 94 days (13.4 weeks, $n = 62$) in the reovirus with chemotherapy arm vs. 50 days (7.1 weeks, $n = 56$) in the chemotherapy alone arm. In the 88 patients discontinued from the study so far the median overall survival was 150 days (21.4 weeks, $n = 50$) in the test arm vs. 115 days (16.4 weeks, $n = 38$) in the control arm. Results of a group of metastatic disease have not yet been published [221, 222].

27.5 Combined Immunotherapy

OVs show their effectiveness in preclinical and clinical studies. However, knowing the immunological basis of tumor biology and the mechanism of OVs action, it should be assumed that the combination of viruses with other immunotherapeutic agents will have a better effect. This is especially relevant for targeting of distant metastatic tumors that are not directly exposed to OV, and accordingly they are not subjected to direct oncolysis and additional stimulation of the immune response with PAMPs, but only immunomediated reactions. In vivo in bilateral flank experiment with implanted human B16 melanoma, Zamarin and co-authors achieved 50% of complete regressions of the primary tumor followed infection with NDV, while the distant tumor that wasn't directly exposed to the virus regressed completely in 20%. In total, long-term survival did not exceed 10%. In the combination of IV NDV with anti-CTLA-4 antibodies (Ipilimumab), the primary tumor was rejected in 90% and the distant tumor in 80% of observations. The long-term survival rate exceeded 70% (in the anti-CTLA-4 group only—no more than 35%) [173].

A combination of vaccinia virus with anti-CTLA-4 antibodies in an experiment with murine models of subcutaneous mouse renal adenocarcinoma and murine colon adenocarcinoma showed an interesting feature of constructing of combined therapy regimens: when the virus and antibodies were administered simultaneously (on day 0), survival and tumor growth rate did not differ from those with vaccinia virus monotherapy, and account for about 10% survival rate by day 30 and tenfold tumor increase on days 20–25. However, administration of antibodies on day 4 from the onset of virotherapy increases survival to about 75% by days 30–35 and reduces the rate of tumor growth—a four- to fivefold increase on day 25. This is attributed to the fact that stimulation of the immunity with anti-CTLA-4 antibodies during the primary replication phase of the virus enhances antiviral immunity (as an increasing amount of CTLs recognizing vaccinia epitopes has been detected in the first case) and does not allow the virus to fully carry out its effect [223].

Reovirus showed increased efficacy when was used in combination with GM-CSF and anti-VEGF. In an experiment with murine tumor models (B16 melanoma), preconditioning with GM-CSF prior to the reovirus injection increased the titer of viral particles in the tumor 100–1000 times through enhancing its delivery to the tumor. An explanation for this is an ability of GM-CSF to mobilize monocyte/macrophages and stimulate infiltration of the tumor with them, which can act as carriers of viral particles. Survival rate of mice preconditioned with GM-CSF was significantly higher than those which undergone administration of either reovirus or GM-CSF alone. It should also be noted that mice that had

antibodies to reovirus showed greater survival and the survival of naive individuals did not significantly differ from control groups [211]. Pretherapy of VEGF-secreting tumors carrying mice with anti-VEGF drugs followed by reovirus administration after 24 h twofold slows murine B16 melanoma tumor growth in the next 30 h compared to anti-VEGF only and to reovirus injected 48 h after anti-VEGF administration. Sunitinib and avastatin, in combination with reovirus, showed a high survival rate of mice, whereas in monotherapy each drug showed a low survival. However, in the same study on the VEGF-non-secreting tumor model, conditioning with the proangiogenic agent $VEGF_{165}$ increased the effect of reovirus and survival twofold. This fact is associated with increased delivery of the virus to a tumor due to the developed tumor vascular system under the influence of $VEGF_{165}$. The authors suggest two scenarios for possible applications of this data: for tumors producing VEGF, a combination of OV with an antiangiogenic agent, and for VEGF-non-secreting tumors—OV with proangiogenic $VEGF_{165}$ [224].

A combination of GM-CSF/reovirus and anti-PD-1 also significantly increases survival compared to GM-CSF/reovirus alone and anti-PD-1 alone in vivo. The same result was observed for a combination of VSV-ASMEL (altered self-melanoma epitope library, engineered VSV) and anti-PD-1. The best effect was shown for a combination of all components: GM-CSF/reovirus/VSV-ASMEL + anti-PD-1. This approach simultaneously covers several aspects of the immune response: GM-CSF/reovirus causes primary oncolysis and release of tumor antigens and stimulates Th1 cells, VSV-ASMEL again provides a spectrum of tumor antigen (ASMEL genes products) and stimulates Th17, and finally anti-PD-1 enhances already activated Th1 and Th17 pools [98].

A combination of T-VEC with ipilimumab in the Phase Ib clinical trial for the treatment of IIIb–IV stage melanoma (with T-VEC regimen as described above, and ipilimumab 3 mg/kg IV every 3 weeks up to totally four infusions starting at the sixth week of virotherapy) showed a satisfactory safety profile with grade 3/4 treatment-related AEs rate of 26.3%, which were mostly associated with ipilimumab. Eighteen-month progression-free survival was 50%, and 18-month overall survival was 67%, which is a better result than when using either T-VEC or ipilimumab as monotherapy [225]. In the Phase II trial of this combination compared with ipilimumab monotherapy, the grade 3/4 AEs rate was 45% and 35% for combination and ipilimumab alone, respectively. Objective response (complete response or partial response, according to the modified immune-related response criteria) was achieved in 39% of patients in the combined therapy arm and 18% in ipilimumab only arm [226].

27.6 Conclusion

Oncolytic virotherapy is a novel stage of the development of cancer immunotherapy. Despite more than a hundred years history of studying various pathogenic agents as a therapy for neoplasms, only with the development of genetic engineering and understanding of the underlying immunological processes of the immunotherapy, their profound study and practical application have become possible. However, there is still a great deal of questions remaining unsolved concerning theoretical and practical aspects of virotherapy, and it cannot be stated that we are close to answering yet.

The immune system plays a central role in realization of the oncolytic potential of viruses. When the cell is infected, stress of the endoplasmic reticulum occurs, which leads to a specific type of death—an immunogenic cell death. During the immunogenic death, the cell secretes pro-inflammatory stimuli that attract innate immune response cells, i.e., NK and dendritic cells. The latter present antigens of the destroyed tumor cell and trigger an adaptive immune response that attacks both the infected tumor and distant, initially uninfected metastatic foci.

The main challenge of adaptation of viruses for their therapeutic use is to increase their selectivity toward tumor cells and to decrease it toward

normal ones. This allows to enhance their effectiveness and to reduce systemic toxicity. Some viruses demonstrate this selectivity naturally and do not require genetic modifications. Mostly these are viruses that are basically nonpathogenic or mild pathogenic for humans: Newcastle disease virus, reovirus, parvovirus, and coxsackievirus. Other viruses require profound modifications, as they normally cause disease in a human or do not show sufficient affinity toward the tumor—HSV, adenoviruses, and vaccinia.

T-VEC (talimogene laherparepvec) is the first oncolytic virus approved by the FDA in 2015 in the United States as a treatment agent for advanced melanoma and in 2016 in Europe and Australia. The drug showed its effectiveness in Phase III trial OPTiM significantly increasing overall survival in comparison with GM-CSF.

Oncolytic adenovirus H101 has been approved in China for the treatment of advanced head, neck, and esophageal tumors. The genome of adenoviruses has been studied quite deeply, and a wide range of different modifications have been proposed for the virus adaptation, even some that allows virus to be activated only in certain types of tissues.

Newcastle disease virus shows its oncolytic properties even without genetic modifications and demonstrates low toxicity even in systemic administration. To date, clinical trial data do not allow us to make a final conclusion about its effectiveness because of the limited number of studies, but the available results clearly indicate the need for further investigation. Nowadays, NDV is being considered mostly in the context of cancer vaccines in the form of viral oncolysates and their various modifications.

Reovirus is currently undergoing Phase III clinical trial as a combined chemo-virotherapy for advanced head and neck tumors. The preliminary results have been published to argue in favor of the effectiveness of the drug.

The combination of oncolytic viruses with other immunotherapeutic agents is the key to enhancing the effect of both, as these drugs potentiate the action of each other. Such combinations remain relatively safe and do not show significant increase in the side effects rates.

Despite the apparent clinical effectiveness of oncolytic viruses and certain successes in understanding the theoretical aspects of their action, much remains not fully defined and contradictory. Further research is needed both for the development of new virotherapeutic agents and for an in-depth understanding of the current ones.

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