Molecular Therapy
Oncolytics
Review

Opportunities and challenges of combining adoptive cellular therapy with oncolytic virotherapy

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The use of oncolytic viruses (OVs) and adoptive cell therapies (ACT) have independently emerged as promising approaches for cancer immunotherapy. More recently, the combination of such agents to obtain a synergistic anticancer effect has gained attention, particularly in solid tumors, where immune-suppressive barriers of the microenvironment remain a challenge for desirable therapeutic efficacy. While adoptive cell monotherapies may be restricted by an immunologically cold or suppressive tumor microenvironment (TME), OVs can serve to prime the TME by eliciting a wave of cancer-specific immunogenic cell death and inducing enhanced antitumor immunity. While OV/ACT synergy is an attractive approach, immune-suppressive barriers remain, and methods should be considered to optimize approaches for such combination therapy. In this review, we summarize current approaches that aim to overcome these barriers to enable optimal synergistic antitumor effects.

INTRODUCTION

Significant advancements have been made in the field of cancer immunotherapy. Adoptive cell therapy (ACT) has been a great technological advancement for leukemias, lymphomas, and myeloma, but there have not been any significant approvals so far in patients with more conventional solid tumors, likely due to a number of barriers in the solid tumor microenvironment (TME). Oncolytic virotherapy has emerged as a promising strategy for specifically infecting and lysing cancer cells, as well as eliciting antitumor immune responses and modulating the TME.[1–3] As such, these two modalities may be complementary for solid tumors. Herein we review the state of the art involving the combination of ACT with oncolytic virotherapy and comprehensively outline strategies with the potential to overcome prospective challenges, particularly in solid tumors.

ADOPTIVE CELLULAR THERAPIES

ACT monotherapy is effective for bone marrow-derived cancers

Apart from the early dendritic cell therapy Provenge for prostate cancer approved over a decade ago, all of the other FDA approvals of ACT have been for hematopoietic cancers (Table 1). Based on our search of www.clinicaltrials.gov, ACT is actively being investigated in over 200 clinical trials. To give the reader a sense of the breadth and scope of those trials, we chose representative active or completed trials to highlight that involve various different types of adoptive cells alone, not in combination with other biological therapies (Table 2). Promising ACTs more recently under investigation include chimeric antigen receptor (CAR)-T cells, T cell receptor (TCR) T cells, tumor-infiltrating lymphocytes (TILs), and both unmodified and modified/expanded natural killer (NK) cells. Thus far, success of cellular therapy as measured by FDA approval has been limited to CD19- and BCMA-targeting CAR-T cells in the setting of hematological malignancies.

ACT monotherapy has shown limited efficacy for solid tumors

Clinical success of ACTs has largely been limited to hematological cancers likely because solid tumors present a number of distinct barriers. Solid tumors have aberrant vascularity as well as complex, dense extracellular matrix (ECM) that potentially limit physical access of blood cells to tumor cells. Furthermore, there are many immunosuppressive factors within the TME.[4,5] Often, immunosuppressive cells such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), regulatory T cells (Tregs), tumor-associated neutrophils, and cancer-associated fibroblasts are adopted by the cancer to maintain immunosuppressive signals and generate physical barriers that mitigate antitumor cellular activity (Figure 1). While next-generation cell therapies are engineered to resist some such immunosuppressive signals such as transforming growth factor β (TGFβ)[6] and to express ECM-degrading enzymes,[7] immunologically cold tumors and distant metastases lack the necessary chemokines to recruit adoptively transferred cells, further constricting ACT effects. Additionally, solid tumors present a paucity of tumor-selective CAR targets, in which target antigens are both specific to and homogeneously expressed on the tumor cells (see review[8]). This heterogeneity places a limitation on CAR approaches, as treatment may initially deplete antigen-positive cells and

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Table 1. FDA-approved adoptive cell therapies for cancers

Product	Description	Indication/use	Date approved
Provenge	PAP-GMCSF-activated CD54+ cells	hormone refractory prostate cancer	Apr 29, 2010
Kymriah	CD19-directed CAR-T cells	refractory B cell precursor ALL (pediatric), refractory after two lines of systemic therapy: DLBCL, high-grade BCL	Aug 30, 2017
Yescarta	CD19-directed CAR-T cells	refractory after two lines of systemic therapy: DLBCL, PMLBCL, high-grade BCL	Oct 18, 2017
Tecartus	CD19-directed CAR-T cells	refractory MCL, refractory B cell precursor ALL	Jul 24, 2020
Breyanzi	CD19-directed CAR-T cells	DLBCL, high-grade BCL, PMLBCL, follicular lymphoma grade 3B	Feb 5, 2021
Abecma	BCMA-directed CAR-T cells	relapsed or refractory multiple myeloma	Mar 27, 2021
Carvykti	BCMA-directed CAR-T cells	relapsed or refractory multiple myeloma after four or more prior lines of therapy	Feb 28, 2022

DLBCL, diffuse large B cell lymphoma; BCL, B cell lymphoma; PMLBCL, primary mediastinal large B cell lymphoma; ALL, acute lymphoblastic leukemia; MCL, mantle cell lymphoma.

contribute to antigen-negative relapse.[9] When considering the setting of combination therapies, understanding potential pitfalls in the dimension of ACT alone is critical in the design of therapies to optimize both safety and efficacy.

ONCOLYTIC VIROTHERAPY

Oncolytic virotherapy as a single agent shows moderate efficacy

The first FDA approval of an oncolytic virus (OV) for cancer was IMLYGIC in October 2015, a modified herpesvirus for treatment of patients with relapsed melanoma, following China's first SDFA approval of Oncorine (H101) for head and neck cancer in November 2005. More recently in 2021, a different oncolytic herpes simplex virus type 1 (HSV) (G47Δ; teserpaturev) was approved in Japan for the treatment of patients with brain tumors. Despite these advances, given the large number of different viruses that have been in clinical trials over the past few decades (Table 3), the relative rate of approvals has been quite low. While the reasons underlying the failures so far are multifactorial, in general OVs as single agents have been less efficacious in humans than in animal models, suggesting they might work best in combination with other therapies, particularly with cellular therapies.

OPPORTUNITIES FOR COMBINING OVs WITH ACTs

OVs have key features to reduce the immunosuppression within the tumor microenvironment and enable an increased immune-cellular response

OVs have recently been recognized for their potential to enhance ACT. They have the capacity to specifically lyse cancer cells, which contributes to immunogenic cell death via release of damage-associated molecular patterns, pathogen-associated molecular patterns, and novel tumor-associated antigens, facilitating antiviral and antitumor responses.[2] In the context of combination therapy, OVs elicit a wave of tumor cell lysis that liberates potential immunogenic tumor-associated antigens, which can result in "epitope spread" wherein endogenous immunity is sensitized to non-ACT target antigens, minimizing the possibility that a tumor can escape all potential

Table 2. List of selected ongoing clinical trials investigating the use of adoptive cell monotherapy

Biological agent	Indication	Status	Sponsor	Trial ID
LN-144 (Lifileucel, autologous TILs, followed by IL-2)	metastatic melanoma	active, phase II	Iovance Biotherapeutics	NCT02360579
LN-145/LN-145-S1, autologous TILs, followed by IL-2)	squamous cell carcinoma of the head and neck	active, phase II	Iovance Biotherapeutics	NCT03083873
MC2 (MAGE-C2/HLA-A2) TCR T cells	melanoma, head and neck cancer	recruiting, phase I/II	Erasmus Medical Center	NCT04729543
huMNC2-CAR44 T cells, autologous	metastatic breast cancer	recruiting, phase I	Minerva Biotechnologies	NCT04020575
HER2/EGFRt-CAR-T cells, autologous	CNS tumors	recruiting, phase I	Seattle Children's Hospital	NCT03500991
MOv19-BBz CAR-T cells	ovarian cancer, fallopian tube cancer, primary peritoneal carcinoma	recruiting, phase I	University of Pennsylvania	NCT03585764
NKX019, allogeneic CD19-CAR NK cells	CD19+ B cell malignancies	recruiting, phase I	Nkarta	NCT05020678
IKDCs (interferon-producing killer dendritic cells), autologous	neoplasm metastasis	completed, phase I	National Defense Medical Center, Taiwan	NCT02661685

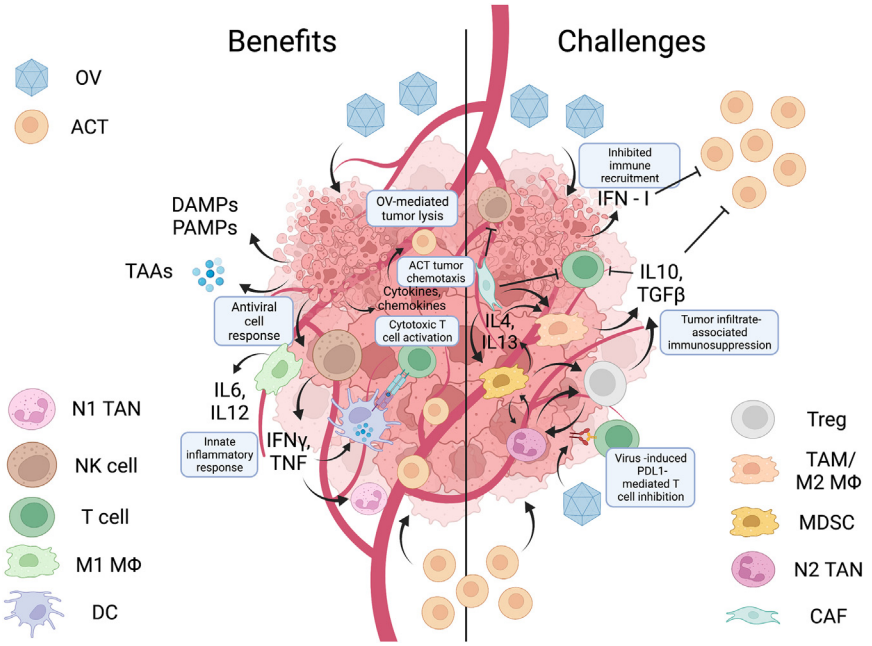

Figure 1. Benefits and challenges of combining oncolytic virotherapies with cellular therapies

Oncolytic viruses have been shown to modify the immunosuppressive tumor microenvironment in many different ways that might enhance the efficacy of cellular therapies, including the production of immune cell chemokines to improve tumor trafficking as well as the induction of proinflammatory cytokines. Some effects may inhibit the function of cell therapies, however, such as the induction of immune checkpoint expression, the recruitment of more myeloid-derived suppressor cells, and the production of immunosuppressive cytokines. Depending on the timing of the two therapies, type I interferons produced by the virus can also inhibit adoptively transferred immune cells.

epitopes, even if it manages to lose the target of the adoptively transferred T cells. OVs also change a previously cold TME to one that actively recruits effector immune cells, thus likely becoming more favorable for a host immune and an ACT antitumor response. OV-induced responses include induction of interferon (IFN) and IFN-inducible chemokines, promoting influx of T cells, NK cells, and dendritic cells to initiate a proinflammatory environment (see review[10]). Stimulation of pathogen recognition receptors not only drives novel chemokine production to promote host-mediated clearance of virus-infected cancer cells, but it concomitantly provides beneficial recruitment of novel immune cells. Additionally, OV-induced neovascularization of the TME[11] may aid in delivery of ACTs to both primary tumor and subsequent metastases, though some OVs paradoxically decrease vascular access.[12]

Challenges to combining OV and ACT for solid tumor therapy

While OV therapy provides potential benefits in enhancing ACT, existing challenges hinder the extent to which OVs can act to provide optimal therapeutic responses in the context of combination therapy. As is a similar barrier to ACT, TME-secreted ECM elements physically prevent ideal OV replication and spread,[13] restricting OV access to pockets within the tumor bed. Tumor sites unperturbed by OVs may prevail and persist to recruit infiltrating immune cells to sustain immunosuppressive signals, mitigating ideal ACT performance. OV-mediated upregulation of programmed death-ligand 1 on cancer cells has been observed,[14–16] which can contribute to immune subversion of ACT. The induction of type I interferons by virus infection, particularly when expressed as a viral transgene, was shown in one study to paradoxically diminish CAR-T antitumor effects by inducing T cell apoptosis and inducing expression of inhibitory receptors, which could be circumvented by knocking out the IFNAR receptor on the CAR-T cells.[17] Furthermore, repetitive administration of OVs may have mitigated efficacy via timely neutralization from acquired antiviral immunity, calling for strategies that can more efficiently subvert the TME to optimize synergy of combination immunotherapies in solid tumors. In addition, ACT itself may limit the spread of OV, requiring carefully timed sequential administration to achieve the best synergy.[18]

While significant work has been done to characterize strengths of OVs and ACTs against cancers, more recent studies have explored the potential of synergizing these two cutting-edge approaches in a multi-faceted approach to enhance therapeutic efficacy beyond the current potential of either approach alone. Despite the field being nascent, efforts to enhance the potential of such combinations are already underway. Investigations of this combination are in early stages, with numerous preclinical investigations published (Table 4) and four phase I clinical trials launched (Table 5).

STRATEGIES TO IMPROVE THE SYNERGY BETWEEN OVs AND ACTs

OV-encoded immune-promoting transgenes

One approach used to enhance ACT involves adjuvant administration of recombinant chemokines and cytokines, which have been shown to enhance therapeutic response via improved intratumoral recruitment and activation of adoptively transferred cells (see review[39]). Examples include the expression from the virus (or a co-injected helper virus) of chemokines such as CCL5, IL12, IL15, and CXCL11 and/or an anti-PDL1 antibody (Table 4). While such activating signals can provide beneficial responses locally, circulating levels can also elicit toxicity. Because they are often administered intratumorally and their replication is generally restricted to cancer cells, OVs can maximize the potential for localized expression of cytokines, chemokines, and/or immune checkpoint inhibitors to tumor sites while minimizing systemic levels to a clinically safe range. With the exception of the previously mentioned expression of type I interferon from the virus, most OVs expressing immunogenic transgenes

Table 3. List of selected ongoing clinical trials investigating the use of OV monotherapy

Biological agent	Indication	Status	Sponsor	Trial ID
Ad5-DNX-2401 (adenovirus)	central nervous system tumors	recruiting, phase I	MD Anderson Cancer Center	NCT03896568
LOAd703 (adenovirus 5/35 encoding TMZ-CD40L and 41BBL)	various carcinomas	recruiting, phase I/II	Lokon Pharma AB	NCT03225989
L-IFN (adenovirus encoding IFN)	various carcinomas	recruiting, phase I	Shanghai Yuansong Biotechnology	NCT05180851
NG-641 (adenovirus encoding CXCL9, CXCL10, IFNa, and FAP-TAc antibody)	metastatic cancer	recruiting, phase I	PsiOxus Therapeutics	NCT04053283
C134 (HSV-1)	central nervous system tumors	recruiting, phase I	University of Alabama	NCT03657576
RP1 (HSV-1)	various carcinomas and melanoma	recruiting, phase I/II	Replimune	NCT04349436
VG161 (HSV-1 encoding IL12/15-PDL1B)	advanced malignant solid tumor	recruiting, phase I	CNBG-Virogin Biotech (Shanghai)	NCT04758897
OH2 (HSV-2)	central nervous system tumors	recruiting, phase I/II	Wuhan Binhui Biotechnology	NCT05235074
GL-ONC1 (vaccinia virus)	advanced solid tumors	completed, phase I	Genelux	NCT00794131
ASP9801 (vaccinia virus encoding IL-7, IL-12)	advanced solid tumors	recruiting, phase I	Astellas Pharma Global Development	NCT03954067
Reolysin (reovirus)	various sarcomas	completed, phase II	Oncolytics Biotech	NCT00503295
MV-s-NAP (measles virus encoding *H. pylori* neutrophil activating protein)	advanced breast cancer	recruiting, phase I	Mayo Clinic	NCT04521764

as monotherapy have demonstrated superior efficacy relative to their "unarmed" counterparts (Table 4), providing groundwork for investigational use for optimizing ACT. One phase I clinical trial investigating the combination of OV + adoptive T cell therapy involves the use of TILT-123, an adenovirus-encoding tumor necrosis factor (TNF) and interleukin-2 (IL-2), in association with T cell therapy using TILs in patients diagnosed with metastatic melanoma.[40]

Viral boosting of adoptively transferred cells

A current challenge of enhancing the potential of ACT lies in the limited ability of adoptively transferred cells to specifically recognize cancer cells. While CAR-based approaches are beneficial in some cancers, they are limited in efficacy relative to homogeneous expression and specificity of CAR targets, particularly in solid tumors. One strategy explores the advantage of tumor-selectivity that OVs provide in order to further enhance antitumor efficacy of ACT. By first loading CAR-T cells with OV *in vitro*, Evgin et al. stimulated *in vivo* expansion of CAR-T cells at the site of the tumor relative to unloaded CAR-T cells, and this strategy was associated with prolonged survival in several mouse models.[37] Furthermore, systemic boosting via additional administration of OV *in vivo*, aimed to propagate activation of adoptively transferred cells against viral or virally encoded epitopes at tumor sites, resulted in >80% cures in mice. This evidence provides rationale for co-administration of ACT cells with pre-loaded OV as well as systemic boosting as a means to promote specific immune activation status at tumor sites.

Modulating the tumor microenvironment via targeting suppressive host immune cells

A major proposed barrier to reaching the potential of most immunotherapies is thought to be the immunosuppressive microenvironment maintained in solid tumors. While mechanisms are not fully understood as to how immune-suppressive cells such as MDSCs, TAMs, and Tregs are sustained in TMEs, inhibition or depletion of such cells in some cases results in impaired tumor growth and induction of antitumor responses.[41–44] Furthermore, targeted depletion of MDSCs and TAMs results in enhanced effects of OV and ACT therapies independently[45,46] Therefore, although speculative, it stands to reason that targeting these immunosuppressive cells in the TME may enhance the combination of OV with ACT, but there are no data yet reported to support that supposition. FDA-approved drugs that reduce or deplete MDSCs and TAMs such as trabectedin, doxorubicin, gemcitabine, lurbinectedin, and indoximod may be useful in this endeavor. Additionally, Goswami et al. recently highlighted small-molecule inhibitors targeting pro-tumor myeloid mechanisms that have demonstrated antitumor efficacy, providing rational combination strategies for solid tumors.[47] Overall, targeted depletion/functional modulation of such cells has a potential to enhance antitumor response in the setting of OV + ACT.

POTENTIALLY MISLEADING IMMUNOLOGIC MODELING

Currently, a major challenge in developmental therapeutics, especially for biologic therapies, is highlighted by their suboptimal success in clinical trials after encouraging results in animal models. One explanation may be that many OVs under investigation differ in their infectivity between species. As a result, not only are the direct lytic effects not seen (or underappreciated) in some animal models, but the effects of a robust infection on immune stimulation are not recapitulated. For example, in our experience, recovery of infectious virus particles following intratumor injection of a variety of different oncolytic human herpes simplex type 1 viruses into mouse tumors (even when implanted into immunodeficient mice) either does not amplify at all or increases only 1–2 logs at best, depending on the model, whereas the same viruses increase 4–5 logs in human xenograft tumors. Thus, depending on the virus and the model, use of fully immune competent models is limited in their ability to emulate the effects of OV infection on ACT activity and may underestimate effects that

Table 4. List of preclinical studies investigating the use of combination OV and adoptive cell therapy

Oncolytic virus	Transgene(s)	Cells	Cancer type(s)	Reference
Adenovirus (Ad5Delta24)	CCL5, IL-15	GD2-CAR-T	neuroblastoma	Nishio (2014)[19]
Vaccinia virus (vvDD) and vesicular stomatitis virus (VSVDeltaM51)	N/A	HER2-CAR-T (loaded with the OV)	breast cancer	VanSeggelen (2015)[20]
HSV-1 (oHSV-1)	N/A	EGFR-CAR NK	breast cancer brain metastases	Chen (2016)[21]
HSV-1 (oHSV-1)	N/A	NK cells	glioblastoma	Yoo (2016)[22]
Adenovirus (Ad5/3Delta24)	IL12, anti-PDL1 expressed by co-injected helper Ad	HER2-CAR-T	head and neck squamous cell carcinoma	Rosewell Shaw (2017)[23]
Adenovirus (Ad5/3Delta24)	Anti-PDL1 expressed by co-injected helper Ad	HER2-CAR-T	prostate, squamous cell carcinoma	Tanoue (2017)[24]
Adenovirus (ICOVIR15K)	EGFRxCD3 bispecific	folate receptor-CAR-T	pancreatic ductal carcinoma/colorectal carcinoma	Wing (2018)[25]
Adenovirus (Ad5/3E2FDelta24)	TNFα, IL-12	mesothelin-CAR-T	pancreatic ductal carcinoma	Watanabe (2018)[26]
Vaccinia virus (vvDD)	CXCL-11	mesothelin-CAR-T	lung cancer	Moon (2018)[27]
Chimeric vaccinia virus (CF33)	truncated CD19	CD19-CAR	breast cancer	Park (2019)[28]
Vesicular stomatitis virus	mIFNβ	mEGFRvIII	murine melanoma	Evgin (2020)[17]
Vaccinia Western Reserve	CCL5	CCR5-NK	various carcinomas	Li (2020)[29]
Adenovirus (Ad5/3Delta24)	CD44v6xCD3 bispecific, IL-12, anti-PDL1 expressed by co-injected helper Ad	HER2-CAR-T	pancreatic ductal adenocarcinoma, squamous cell carcinoma	Porter (2020)[30]
Adenovirus (rAd.sT)	TGFb decoy	mesothelin-CAR-T	breast cancer	Li (2020)[31]
Vaccinia virus	CD19	CD19-CAR-T	Melanoma	Aalipour (2020)[32]
Adenovirus	CD19 tag	CD19-CAR-T	liver cancer	Tang (2020)[33]
Adenovirus (Ad5/3Delta24)	IL12, anti-PDL1 expressed by co-injected helper Ad	HER2-CAR-T	pancreatic cancer	Rosewell Shaw (2021)[34]
Vesicular stomatitis virus (VSVDeltaM51)	IL-15	NKT	pancreatic cancer	Nelson (2022)[35]
HSV-1 (G47Δ)	N/A	Lp2-CAR-T	Glioblastoma	Chalise (2022)[36]
Vesicular stomatitis virus, reovirus	mIFNβ (VSV)	mEGFRvIII	murine melanoma, glioma	Evgin (2022)[37]
HSV-1	OX40L, IL-12	TILs	colon cancer, pancreatic cancer	Ye (2022)[38]

might be result from a robust virus replication in humans. On the other side, the use of xenografts in immunodeficient mice does allow study of the immunologic response to viruses, and may over estimate the oncolytic effects as the infection is not constrained by immunity. These concerns may be mitigated somewhat by the use of human tumor xenografts in bone marrow humanized mice as was used in at least one study,[34] though immune cells and tumors in that setting expressed mismatched major histocompatibility complex, which could also artificially impact the results.

Another major area of disconnect that might underly differences between animal models and patient outcomes lies in the immunological differences between humans and preclinical models. As highlighted by Mestas and Hughes,[48] proportional differences in immune cell

Table 5. Combination OV and adoptive cell clinical trials for cancers

Biological agent	Combination	Indication	Status	Sponsor	Trial ID
TILT-123 (adenovirus coding TNFα and IL2)	adoptive cell therapy with TILs	metastatic melanoma	recruiting, phase I	TILT Biotherapeutics	NCT04217473
CAdVEC (binary oncolytic adenovirus)	HER2-specific CAR-T cells	advanced solid tumors	recruiting, phase I	Baylor College of Medicine	NCT03740256
VCN-01 (oncolytic adenovirus expressing hyaluronidase)	mesothelin-specific CAR-T cells	pancreatic cancer, serous ovarian cancer	recruiting, phase I	University of Pennsylvania	NCT05057715
OVV-01 (oncolytic vaccinia virus)	trained immunity NK cells IBR900	advanced solid tumors including lymphoma	terminated	Beijing Boren Hospital	NCT05271279

subsets, toll-like receptor differences, and varying levels of cytokine responses have been noted between humans and mice. Furthermore, many mouse cytokines and chemokines do not cross-react with their human counterparts and vice versa. For example, while type I interferons are potent activators of antiviral and immunoregulatory responses,[37] no appreciable cross-reactivity is seen between human and mouse type I interferons.[48] In human xenograft models, this alone would detract from biologically relevant cross-talk—resulting in lack of innate antiviral responses—potentially providing an overestimate of true viral permissivity by allowing an "artificially" extended viral spread and reduced antitumor immune response relative to the therapeutic response that would be found in human patients. Thus, in the ongoing investigation for biological therapeutic approaches, it may be critical to implement models on various axes of interaction to more closely mimic those that occur in human patients. While current advancements of such humanization methods largely include immune cell engraftment, as highlighted in a recent review,[49] the use of supplementing human versions of chemokines and cytokines expressed from an OV within a humanized model may also uniquely serve to mimic responses seen in humans.

CONCLUSIONS

There are numerous biologic rationales for combining adoptive cellular therapy with oncolytic virotherapy, and preclinical efficacy looks promising in some studies but is cross-inhibitory in others. Many unknowns still need to be investigated, however, including relative dosing, timing, and engineering of each to overcome mitigating factors when these two promising therapies are combined. Better preclinical models that more accurately recapitulate complex human immune cell interactions are needed in order to improve our success rates in clinical translation. Focusing on these challenges will be pivotal for fully realizing the potential of ACT + OV combinations.

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AUTHOR CONTRIBUTIONS

J.A.M., C.Y.C., M.A.C., and T.P.C. wrote the manuscript, and K.C., D.A.L., and T.P.C. edited the manuscript. J.A.M. drew the figure.

DECLARATION OF INTERESTS

D.A.L. is an inventor on patents in cellular therapy and has licensed related technology to Sanofi. The other authors are inventors on patents or pending patents regarding oncolytic virotherapy. T.P.C. has licensed one such OV technology to Vironexis Biotherapeutics, Inc.

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