

University of Groningen

Oncolytic virotherapy - analysis, design, models

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DOI:
[10.33612/diss.859671389](https://doi.org/10.33612/diss.859671389)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2024

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Bhatt, D. (2024). *Oncolytic virotherapy - analysis, design, models*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.859671389>

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CHAPTER 2

A systematic analysis on the clinical safety and efficacy of Onco-Virotherapy

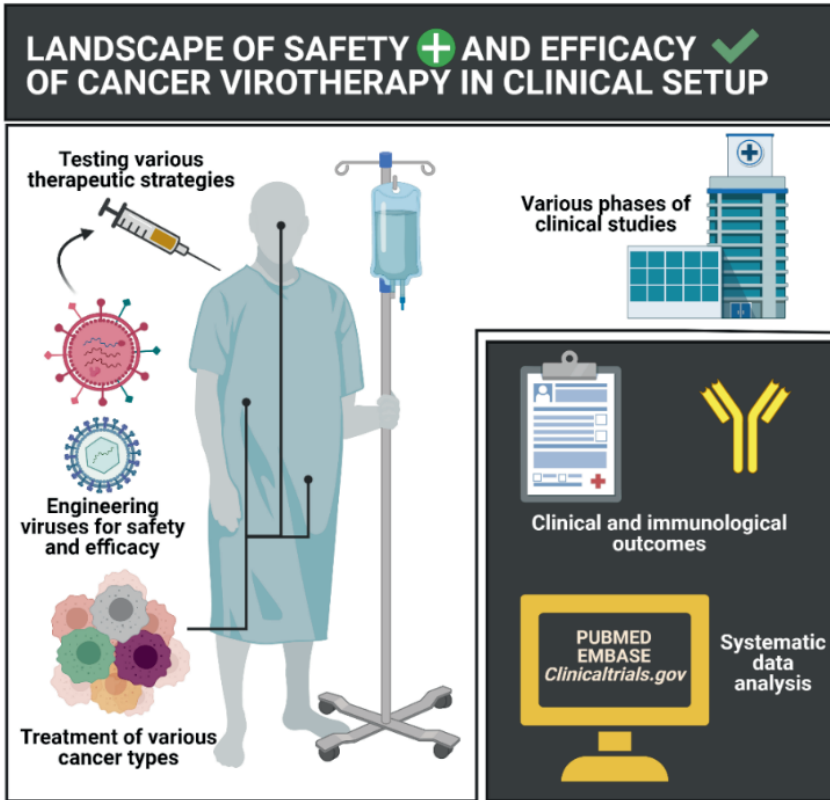
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Published in Mol Ther Oncolytics. 2021



Abstract

Several onco-virotherapy candidates have been developed and clinically evaluated for the treatment of cancer, and several are approved for clinical use. In this systematic review we explored the clinical impact of onco-virotherapy compared to other cancer therapies by analysing factors such as trial design, patient background, therapy design, delivery strategies and study outcomes. For this purpose, we retrieved clinical studies from three platforms: *clinicaltrials.gov*, PubMed and EMBASE. We found that most studies were performed in patients with advanced and metastatic tumors, using a broad range of genetically engineered vectors and mainly administered intratumorally. Therapeutic safety was the most frequently assessed outcome, whilst relatively few studies focused on immunological antitumor responses. Moreover, only 59 out of 896 clinical studies were randomized controlled trials reporting comparative data. This systemic

review thus reveals the need of more, and better controlled, clinical studies to increase our understanding on the application of onco-virotherapy either as single treatment or in combination with other cancer immunotherapies.

Keywords: oncolytic virotherapy, cancer, clinical study, trial, outcomes, immune response, systematic review

Introduction

In the last two decades, viral vector-based therapies are gaining increasing attention as a promising strategy for cancer treatment. Studies in the field of cancer virotherapy have explored the administration of viral vectors as agents for therapeutic vaccines ¹, gene therapy ²⁻⁴, and more recently as oncolytic therapeutics ^{5,6}. To date, several viral vectors are approved for clinical application. Safety and efficacy are the primary goals of clinical trials and therefore, the clinical success of cancer virotherapy depends on these outcomes. Advanced genetic engineering tools have allowed researchers to improve safety by enhancing tumor targeting and tumor replication ⁵. Additionally, with these tools, the efficacy of viral vectors for onco-virotherapy can be enhanced by encoding transgenes that strengthen the oncolytic potential or that elicit stronger antitumoral immune responses⁷. Besides vector design, factors as clinical trial design, patient background, dose, frequency, delivery strategy, issues related to immune-mediated virus elimination, and the choice of clinical outcome measures may also influence clinical success. In this study we aimed to analyse these parameters based on a systematic review. For this purpose we retrieved articles from several platforms in the context of onco-virotherapy for antitumor responses. Previous systematic reviews on onco-virotherapy were based on a limited number of articles retrieved exclusively from PubMed ^{6,8}, thereby overlooking studies archived by other platforms, while a recent review focused on randomized controlled trials only ⁹. To provide a broader overview of the global trends in clinical research related to onco-virotherapy we conducted an extensive literature survey that includes a more complete set of articles and trials retrieved from multiple platforms, including *clinicaltrials.gov*, PubMed (Medline), and EMBASE.

For a comprehensive analysis of the clinical research in onco-virotherapy to date, our dataset includes phase I-IV trials, along with cohort- and case studies.

Materials and Methods

Protocol and eligibility: We used the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocol (PRISMA-P) statement as a guide for our analysis. To define our research question, we utilized the PICOS (Patient, Intervention, Comparator, Outcome, Study type) framework based on the accepted PRISMA guidelines. Accordingly, we focused on cancer patients (P), that receive onco-virotherapy (I), compared with patients that receive other therapies (including placebo, chemotherapy, immunotherapy, radiotherapy) (C), the clinical impact w.r.t. response rate (O1) or tumor size change (O2) or safety (O3), etc. (O4-to-n), and in a clinical setup (S) for therapeutic purposes.

Search strategy and screening of articles: We retrieved clinical trials from the *clinicaltrials.gov* registry (<https://clinicaltrials.gov>) and PubMed (<https://pubmed.ncbi.nlm.nih.gov>) and EMBASE (<https://www.embase.com>) databases until August 2020. For each medium, we used a different search strategy, as specified in the supplementary information. Through the search strategy, we found 3492 articles from PubMed, 2614 articles from EMBASE, and 1632 trials from *clinicaltrials.gov*. After the removal of duplicates, at least two authors manually screened the retrieved articles for inclusion, where we excluded articles or trials that did not focus on the application of onco-virotherapy for cancer patients, articles that were reviews, preclinical studies or commentaries, and articles in which the abstract was not reported in the English language (figure 1). Any conflicts were resolved through discussion. This allowed us to include 331 articles from PubMed, 316 articles from EMBASE and 249 trials from *clinicaltrials.gov*. Subsequently, we added all articles in our database using browser-based REDCap software (Vanderbilt University, Nashville, USA). All the data and results are provided in supplementary material Table 1 and are intended to serve as a resource for future studies.

Preliminary qualitative analysis and screening of controlled-clinical studies: To observe the trends in clinical studies exploring the potential of onco-virotherapy for cancer treatment, we did a preliminary analysis of studies

including retrieved data from articles and trials (figure 1 to 5). This preliminary analysis was based on the literature found via PubMed (331 articles), EMBASE (316 articles), and *clinicaltrials.gov* (249 trials) as described earlier. Finally, we identified and summarized 59 controlled clinical studies reporting comparative data from respective articles and trials (table 1). All figures and tables were made using ggplot2, or networkD3 R packages ¹⁰, and the graphical abstract was made using Biorender.

Results

Scenario of clinical studies evaluating onco-virotherapy

A systematic search performed on PubMed, EMBASE, and *clinicaltrials.gov*, retrieved until August 2020, found 249 trials, 331 articles on PubMed, and 316 articles on EMBASE, that contained relevant terms (supplementary information) and fulfilled the necessary inclusion criteria required for the analysis on clinical data related to onco-virotherapy (figure 1A). Of these trials and articles, 59 entries contained data from controlled clinical trials, allowing the comparison of onco-virotherapy with either placebo, standard palliative care, or conventional therapy (figure 1A). Most of these studies were performed in North America, the Republic of China and Europe (figure 1B).

There has been an increase in the number of studies in the past two decades, which can be attributed to the widespread availability of genetic engineering platforms and molecular techniques to design and test onco-virotherapy in both pre-clinical and clinical stages (figure 2A). Especially the approval of Talimogene laherparepvec¹¹ (T-VEC, herpes virus with infected cell protein (ICP) 34.5 and ICP47 deletion, encoding granulocyte-macrophage colony-stimulating factor (GM-CSF)) by the FDA and EMA in 2015, revived the interest for clinical applications of onco-virotherapy (figure 2A). Most clinical studies have been conducted at phase I and II stage, often to test the safety and maximum tolerated dosage of the onco-virotherapy (figure 2B). Although a wide range of viral-vectors have been tested in phase I and II trials for safety⁵, few studies have progressed further to phase III trials (figure 2B). In terms of the genetic nature, both enveloped and non-enveloped DNA and RNA vectors have been tested. Adenovirus (non-enveloped

DNA virus) was the most commonly studied platform with 42.5% studies, followed by herpes simplex virus (enveloped DNA virus) with 21.3% studies, 13.2% studies of vaccinia virus (enveloped DNA virus), and 7.3% studies of reovirus (non-enveloped RNA virus). Patients with advanced and metastatic tumors were the most frequently recruited patients to receive onco-virotherapy (figure 2C), likely due to the fact that cancer patients with a good prognosis generally benefit from standard care. However, this may limit our understanding of the potential efficacy of onco-virotherapy in patients with early-stage cancers.

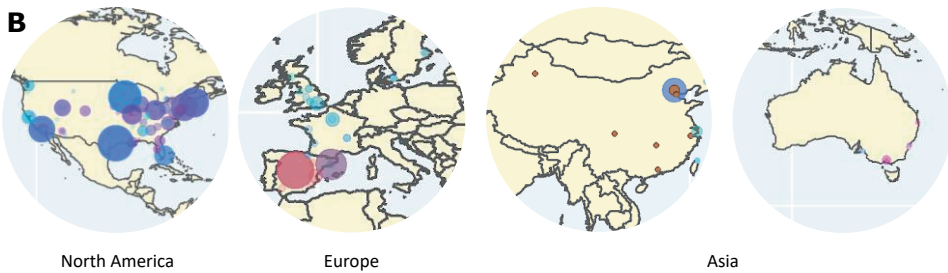
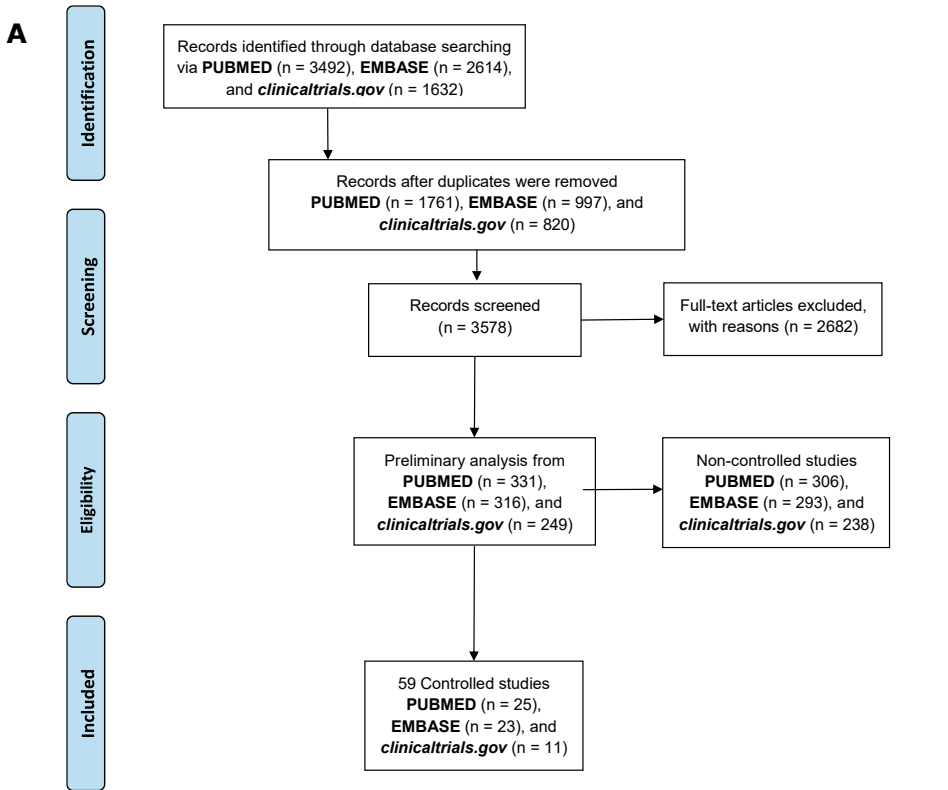


Figure 1: Screening of studies focusing on clinical safety and efficacy of onco-virotherapy. (A) Systematic review process and inclusion of articles and trials based on target criteria, where excluded reports did not focus on the application of onco-virotherapy for cancer patients, or were reviews, preclinical studies or commentaries, and articles in which the abstract was not reported in the English language, (B) Geographical distribution of labs and institutes assessing safety and efficacy of onco-virotherapy.

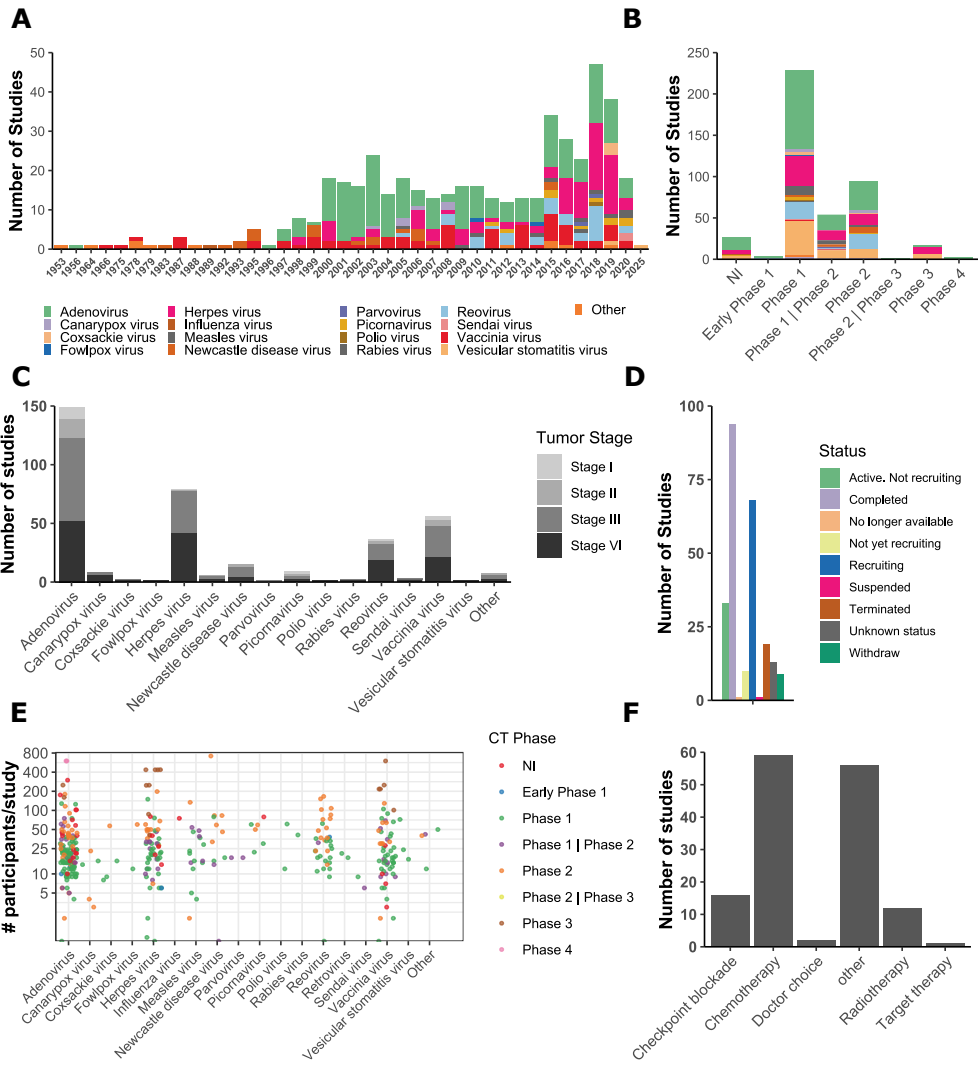


Figure 2: Scenario of clinical studies assessing onco-virotherapy. (A) Trends in clinical studies published as trials and articles assessing the role of different onco-virotherapy. (B) Frequency of studies published as trials and articles according to phase and type of onco-virotherapy studied, legend the same as in 'a'. (C) Patient tumor-stage and status that received onco-virotherapy, (D) Onco-virotherapy trial status as per clinicaltrials.gov, (E)

Number of cancer patients recruited per study and treated via onco-virotherapy in different phases, (f) Frequency of therapeutic combination with onco-virotherapy.

Although more than 200 trials, related to onco-virotherapy, are registered on *clinicaltrials.gov*, less than 100 trials have been completed (figure 2D). Many studies have reported being terminated or suspended due to funding issues or a lack of participants, and some trials are still active or recruiting patients. More than 2000 cancer patients have been recruited and treated with onco-virotherapy, with phase I/II trials mostly conducted in a relatively small group of patients, and phase III trials with more than 200 patients per group (figure 2E). Onco-virotherapy has been given to patients as a monotherapy, whilst, occasionally it has been combined with chemotherapy and radiotherapy, and in some cases with targeted therapy (figure 2F). Moreover, a limited number of case studies that did not have success with conventional checkpoint therapy or radio/chemotherapy (indicated as doctor's choice in figure 2F) later proceeded with onco-virotherapy alone¹² or in combination with cyclophosphamide¹³ to treat recurrent tumors in patients. Recent preclinical findings supporting the combination of immunotherapy have also led to clinical studies where checkpoint inhibitors targeting PD-1 (programmed death receptor-1) or PDL-1 (programmed death ligand-1) have been administered along with onco-virotherapy ¹⁴.

Viral modifications and strategic therapy design to improve safety and efficacy

To establish the safety of onco-virotherapy for cancer-patients, genetic modifications have been performed on a wide range of viral vectors to improve tumor targeting and -attachment or to enhance tumor-specific replication (figure 3A). Adenoviruses, herpes viruses, vaccinia viruses, and reoviruses have often been engineered to improve tumor specificity by such modifications. Improvement in targeting of adenovirus was for example achieved through knob modifications, and attachment was improved by adenoviral fiber protein delta-24-RGD modification or via intercellular adhesion molecules. Alternatively, viral replication was restricted to tumor cells through modification or deletion of viral proteins such as early proteins (E1 to E4) in adenovirus, and deletion of ICP34.5 and ICP47 in herpes virus (supplementary table 1). Moreover, in some cases reovirus, vaccinia

virus and adenovirus were designed to have target-specific replication in tumor cells with differentially activated pathways such as RAS GTPases (rat sarcoma GTPases) or p16-RB (retinoblastoma protein) pathways (supplementary table 1). The 2018 Nobel prize-winning technique of directed evolution has also been implemented as a means to screen adenoviruses with improved selectivity for tumor cells and their subsequent oncolysis.

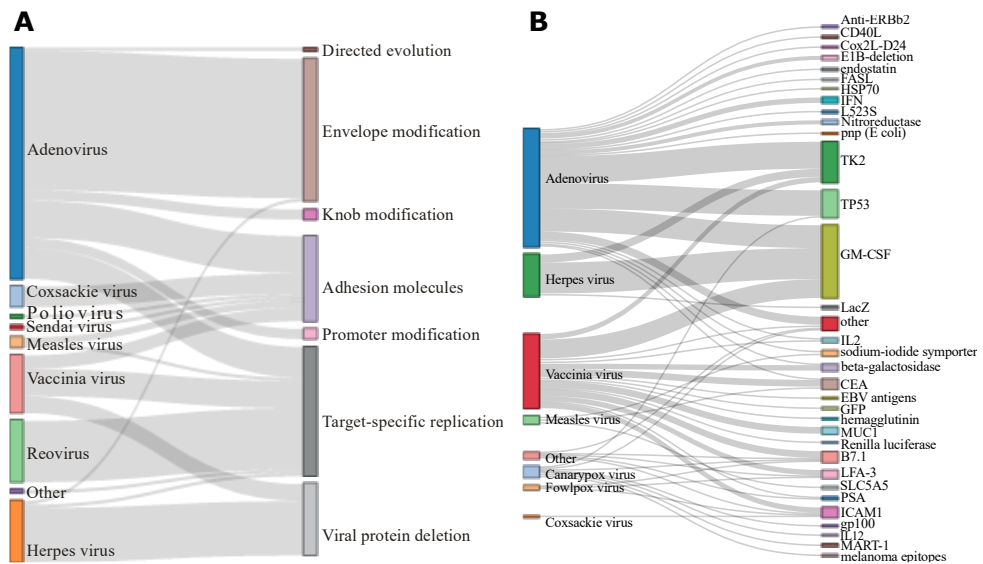


Figure 3: Viral modifications to improve safety and efficacy. (A) Virus modifications to improve tumor specificity. (B) Introduction of transgenes to improve therapeutic efficiency. Each line represents a single study (trial or article).

Viral vectors have also been engineered to deliver and encode transgenes that act as 'suicide-genetic switch' for controlled lysis of target cancer cells, for example by using ganciclovir to induce cell-death of tumor cells expressing thymidine kinase (TK2) transgene (figure 3B). Simultaneously, viral vectors have been modified to improve efficacy by incorporating genes to enhance or direct antitumor immune responses. These are either tumor-specific antigens like prostate specific antigen (PSA) and mucin1 (MUC1), antitumor genes including tumor suppressor protein 53 (TP53), and genes encoding growth factors, cytokines or ligand molecules, such as GM-CSF, interferon, interleukin-12, fas-ligand and CD40-ligand, or marker genes encoding fluorescent proteins or enzymes that can be used for detection and quantification of transgene expression such as galactosidase and luciferase (figure 3B). The most commonly encoded

genes were found to be GM-CSF, TP53, and TK2, while adenoviruses, herpes viruses, and vaccinia viruses were the most frequently engineered vectors of choice.

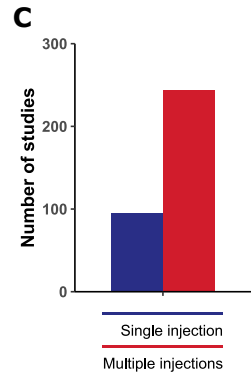
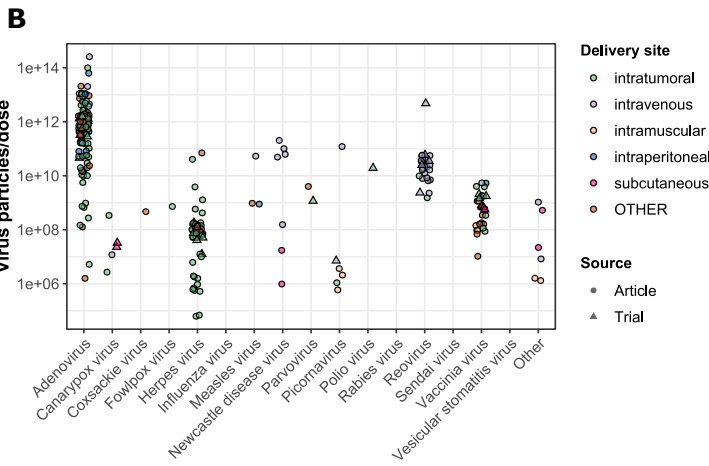
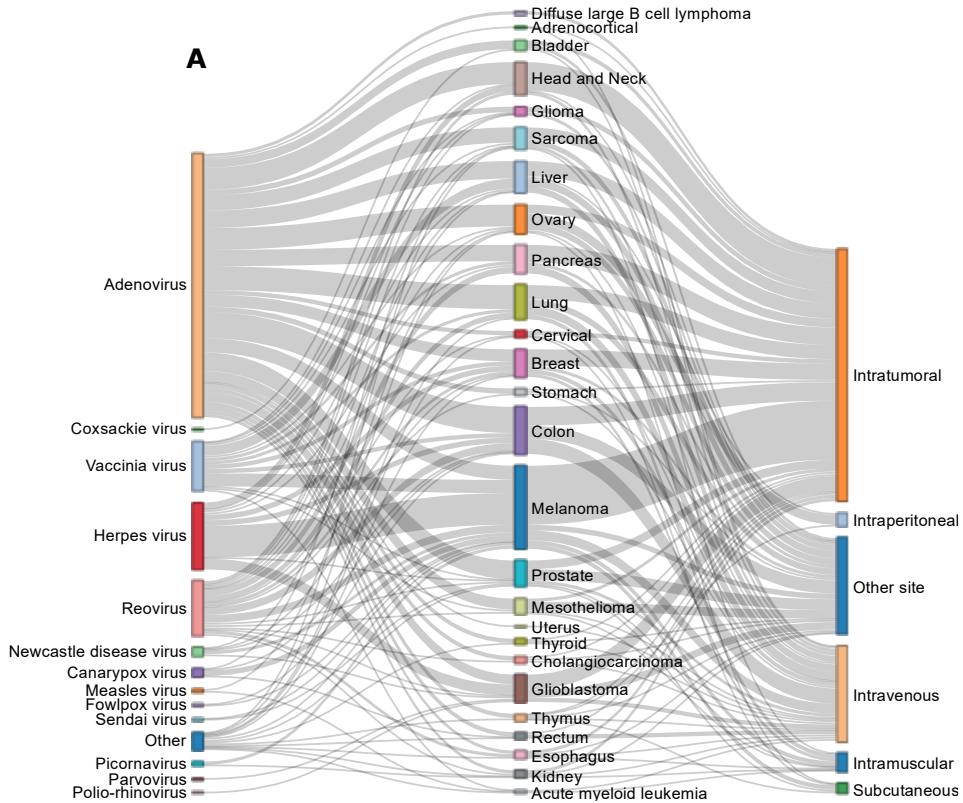


Figure 4: Strategic design to improve safety and efficacy. (A) Trends in choice of viral vector and delivery site according to tumor type. Each line represents a single study (trial or article), (B) Maximum tolerated dose per virus type in patients, (C) Frequency of injections applied.

Regarding therapeutic delivery, intratumoral delivery of viral vectors has remained the preferred route of injection due to safety and efficacy concerns by restricting viral infection to tumor. Nevertheless, intravenous, subcutaneous, and intramuscular routes have also been tested to achieve better biodistribution and to target distant metastatic sites (figure 4A). In the case of melanoma, intratumoral delivery has remained a preferred choice due to easier accessibility of tumors. A wide range of virus doses have been tested for each viral vector type, and safety has been associated with lower doses, albeit at the cost of therapeutic efficacy. For example, adenoviruses have been given to patients at doses considered safe up to 10^{14} particles per injection for best efficacy, whereas herpes viruses have demonstrated to be efficient in the range of 10^6 to 10^8 particles per injection (figure 4B). In terms of the number of virus injections given to cancer patients, multiple injections were preferred and the scheme varied from daily, weekly, and monthly intervals (figure 4C and supplementary table 1).

Evaluation of clinical outcomes related to safety and efficacy

To test the therapeutic efficacy of viruses, studies have assessed different clinical outcomes like overall survival, tumor size change, and overall response rate. However, as most of the trials comprised phase I/II stages, the most common study outcome was safety (figure 5A). Although onco-virotherapy is nowadays also considered to induce antitumor immune responses, there have been relatively few (or have been fewer) studies assessing immunological outcomes (figure 5A). Interestingly, the most commonly studied immunological features were antibody responses to viral vectors and antitumor adaptive responses mediated via lymphocytes (figure 5B). Immune responses related to myeloid cells have been rarely assessed in clinical studies^{6,15-20}, which might be due to difficulties in obtaining and processing clinical tissue samples from the patients as compared to the easier accessibility of peripheral blood to study lymphocytes, antibodies, and cytokine-based innate responses (figure 5B).

Considering the controlled clinical trials, onco-virotherapy has often been compared with standard palliative care and/or treatment in addition to placebo groups of patients (figure 5C). In the case of combinatorial therapeutic approaches, the control group was treated with virus alone. Onco-virotherapy, as compared to these standard treatments, either resulted in a better or similar outcome but rarely worsened the outcome as based on overall survival, progression-free survival and decrease in tumor size (figure 5D).

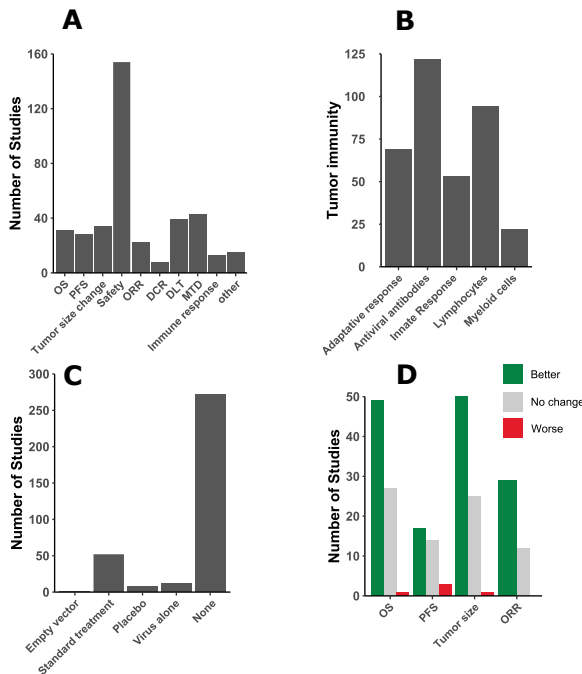


Figure 5: Clinical Outcomes studied related to efficacy. (A) Frequent clinical outcomes studied post-onco-virotherapy, (B) Type of immunological outcomes studied, (C) Commonly assessed control groups in comparison to onco-virotherapy, (D) Significant improvement or not in clinical outcomes post onco-virotherapy as compared to respective control groups. OS: Overall survival, PFS: Progression free survival, ORR: Objective response rate, DCR: Disease control rate, DLT: Dose limiting toxicity, MTD: Maximum tolerated dose.

The factors related to each of the controlled trials are summarized in table 1. Of note, most trials did not involve control groups. Yet, also many of the trials with control groups had major limitations as onco-virotherapy had to be compared to standard treatment or onco-virotherapy plus standard treatment. For example, in some cases the standard treatment was palliative care, placebo therapy or observational data from tumor-type matched patients which does not provide an indication of the improvement due to onco-virotherapy in comparison to conventional chemo- and radiotherapy. Differences in individual trial design and multitude of outcome measures make it difficult to compare studies performed by independent-unrelated institutes. Also, many articles and clinical trials have incomplete descriptions of the methods employed, increasing the difficulty of making comparisons and meta-analysis even more.

Table 1: Summary of controlled clinical trials exploring safety and efficacy of onco-virotherapy.

Study	Virus type	Dose	Tumor type and stage	Mean age and gender	Control group	Transgene encoded	Tumor Specificity	Follow up (months)	End point	Outcome
1975, Everall J.D. ²¹	Vaccinia virus	1 ⁸	Melanoma, stage 1	49.5 years, both	Wide local excision	NA	NA	48	Progression free survival	No change
1989, Freedman R.S. ²²	Influenza A virus	NA	Uterine cervix carcinoma	46 years, female	Radiotherapy	NA	NA	95	Progression free survival	No change
1992, Schlag P. ²³	Newcastle disease virus	1 ⁷	Colorectal cancer with metastasis to the liver, stage 4	55 years, both	Surgery	NA	NA	NA	NA	NA
1993, Csatory L.K. ²⁴	Newcastle disease virus	NA	Various cancers, stage 3	NA, both	Placebo	NA	NA	24	Overall survival	Better
1995-96, Ockert D. ²⁵	Newcastle disease virus	NA	Colorectal carcinoma	NA, both	Surgery	NA	NA	22	Safety	Safe
1995, Htnkel A. ²⁶	Newcastle disease virus	2 ⁶	Renal cell carcinoma, stage 3-4	NA, both	Uninfected irradiated renal carcinoma cells, virus alone, IL2 alone	NA	NA	NA	NA	NA
1995, WalLack M.K. ²⁷	Vaccinia virus	NA	Melanoma, stage 2	NA, both	Vaccinia virus alone	NA	NA	48	Progression free survival	No change
1997, WalLack M.K. ²⁸	Vaccinia virus	NA	Surgically resected melanoma, stage 2	NA, both	Vaccinia virus alone	NA	NA	42.28	Overall survival	No change
1998, WalLack M.K. ²⁹	Vaccinia virus	NA	Melanoma, stage 3	NA, both	Vaccinia virus alone	NA	NA	46.3	NA	No change



Systematic analysis on clinical safety and efficacy of Onco-virotherapy

2000, Sandmair A.M. ³⁰	Adenovirus	3 ¹⁰	Glioma, stage 3-4	51 years, both	LacZ galactosidase	NA	NA	15	Safety	Safe
2000, Ratnov N.G. ³	Herpes virus	NA	Newly diagnosed, previously untreated glioblastoma multiforme	59.3 years, both	Surgical resection and radiotherapy	Thymidine kinase 2	Deletion ICP34.5 and ICP47	NA	Progression free survival	No change
2002, Habib N. ³¹	Adenovirus	3 ¹¹	Hepatocellular carcinoma, stage 2	59 years, both	Percutaneous ethanol injection	NA	E1B-deletion	1	Safety	Safe
2003, Voit C. ³²	Newcastle disease virus	NA	Melanoma, stage 3	53.5 years, both	Placebo	NA	NA	18	Safety	Safe
2003, Zhang S. ³³	Adenovirus	1 ¹²	Head and Neck Squamous Cell Carcinoma	NA	Radiotherapy	TP53	NA	NA	Tumor size	Better
2003, Chen C. ³⁴	Adenovirus	NA	Nasopharyngeal carcinoma	NA, both	Radiotherapy	TP53	NA	3	Safety	Safe
2004, Xia Z.J. ³⁵	Adenovirus	1.5 ¹²	Squamous cell cancer of head and neck or esophagus	NA, both	Cisplatin with 5-fluoro-uracil or Adriamycin with 5-fluoro-uracil	NA	E1B-55kDa gene deletion	NA	Objective response rate	Better
2006, Spaner D.E. ³⁶	Canarypox	5 ⁶	Melanoma, stage 3-4	50 years, both	Antigen peptides alone	gp100 antigen	NA	8	NA	NA
2006, Lindsey K.R. ³⁷	Vaccinia virus	2 ⁹	Melanoma, stage 3-4	47 years, both	Virus alone	Tyrosinase	NA	4	NA	No change

2008, NCT00613509	Canarypox	NA	Melanoma, stage 3-4	52.8 years, both	Interferon alpha-2b	Multimelanoma antigens	NA	88	Progression free survival	Better
2008, Dong M. et al. ³⁸	Adenovirus	2 ¹²	Lung, Ovarian, Liver, Breast, Celiolthelioma, stage 3-4	59 years, both	Cisplatin	TP53	NA	2	Objective response rate	Better
2009, Guan J. ³⁹	Adenovirus	1 ¹²	Non-small cell lung cancer, stage 3-4	58 years, both	Bronchial arterial infusion	TP53	NA	12	Safety	Safe
2009, Pan J.J. ⁴⁰	Adenovirus	1 ¹²	Nasopharyngeal carcinoma, stage 2-4	48.5 years, both	Radiotherapy	TP53	NA	72	Objective response rate	Better
2009, Tian G. ⁴¹	Adenovirus	1 ¹²	Hepatocellular carcinoma	55.5 years, both	Transcatheter arterial chemoemboliz ation	TP53	NA	128	Safety	Safe
2010, Yang X. ⁴²	Adenovirus	3 ¹²	Hepatocellular carcinoma	55 years, both	Fractionated sterotactic radiotherapy	TP53	NA	35	Safety	Safe
2010, NCT01280058	Reovirus	NA	Pancreatic cancer, stage 4	64 years, both	Carboplatin with paclitaxel	NA	RAS proto- oncogene dependency	48	Progression free survival	Worse
2011, Heo J. ⁴³	Vaccinia virus	1 ⁹	Liver cancer	47.6 years, male	Historical data of control patients or Sorafenib alone	GM-CSF	EGFR-Ras dependency	2	Tumor size	Better
2011, Cerullo V. ⁴⁴	Adenovirus	1 ¹²	Advanced metastatic solid tumors, stage 3-4	61 years, both	Cyclophospha mide in combination with virotherapy	GM-CSF	RGD-D24 targeting	12	Overall survival	Better
2012, Koski A. ⁴⁵	Adenovirus	1 ¹¹	Colorectal, sarcoma, pancreatic,	57.5 years, both	Verapamil	GM-CSF	Integin targeted	NA	Overall survival	No change

Systematic analysis on clinical safety and efficacy of Onco-virotherapy

		lung, breast, mesothelioma	Ad5-D34-RGD							
2013, <i>Suriano R.</i> ⁴⁶	Vaccinia virus	Melanoma, stage 3	NA	50 years, both	NA	NA	NA	NA	Overall survival	No change
2013, <i>Westphal M.</i> ⁴⁷	Adenovirus	High-grade glioma, stage 3	1 ¹²	55.4 years, both	Resection and standard care	Thymidine kinase 2	NA	152	Overall survival	No change
2014, <i>Dong J.</i> ⁴⁸	Adenovirus	Unresectable hepatocellular carcinoma, stage 3-4	1 ¹²	54 years, both	Transarterial chemoembolization alone	NA	NA	NA	Progression free survival	Better
2014, <i>Freytag S.O.</i> ⁴⁹	Adenovirus	Intermediate risk prostate cancer, stage 2	1 ¹²	61 years, male	Radiotherapy	Thymidine kinase 2	NA	48	Safety	Safe
2015 and 2018, <i>NCT00769704</i>	Herpes virus	Melanoma, stage 3-4	4 ⁸	64 years, both	GM-CSF therapy	GM-CSF	Deletion of ICP34.5 and ICP47	44.4	other	NA
2015, <i>Donina S.</i> ⁵⁰	Picornavirus	Melanoma, stage 1-2	1 ⁶	62.3 years, both	Untreated observational group	NA	NA	47.8	Overall survival	Better
2015, <i>NCT00769703</i>	Herpes virus	Melanoma, stage 3-4	1 ⁸		GM-CSF therapy	GM-CSF	Deletion of ICP34.5 and ICP47	44	Disease control rate	Better
2015, <i>Kanerva M.</i> ⁵¹	Adenovirus	Various cancer	NA	60.5 years, both	Matched controls y cancer type and disease phase	GM-CSF	Integrin-targeted	46	Safety	Safe
2015, <i>Lin X.</i> ⁵²	Adenovirus	Hepatocellular carcinoma	1 ¹²	55 years, both	Transarterial chemoembolization of carboplatin	NA	E1B-deletion	12	Overall survival	Better

2015, NCT00179309	Vaccinia virus	1 ⁹	Breast cancer, stage 4	54.3 years, both	Docetaxel	CEA, MUC1 and TRICOM	197	Progression free survival	Better
2015, NCT00634595	Adenovirus	1 ¹²	Head and Neck Squamous Cell Carcinoma, stage 3-4	52 years, both	Cisplatin and Paclitaxel	Endostatin	10	NA	No change
2016, NCT02705196	Adenovirus	NA	Pancreatic cancer, stage 3	>18 years, both	Gemcitabine and Paclitaxel +/- anti-PDL1 antibody	NA	NA	NA	NA
2016, Andtbacka R. H. I. ^{11,53}	Herpes virus	NA	Melanoma, stage 3-4	63 years, both	Subcutaneous injection of GM-CSF	Deletion of ICP34.5 and ICP47	NA	Tumor size	Better
2016, Gao Y. D. ⁵⁴	Adenovirus	5 ¹⁰	Malignant and solid tumors, stage 3-4	35 years, both	Adriamycin alone	GM-CSF	NA	NA	Better
2016, NCT00870181	Adenovirus	1 ¹²	High-grade gliomas, stage 3-4	52.5 years, both	Surgery, systemic chemotherapy or palliative care	Thymidine kinase 2	71	Progression free survival	Better
2016, Andtbacka R. H. I. ^{11,53}	Herpes virus	1 ⁸	Unresected melanoma, stage 3-4	63 years, both	GM-CSF therapy	Deletion of ICP34.5 and ICP47	30	Disease control rate	NA
2016, Su X. ⁵⁵	Adenovirus	1 ¹²	Cervical cancer. Stage 2-3	52 years, female	Radiotherapy in combination with brachytherapy	TP53	605	Safety	Safe
2016, Harrington et al ⁵⁶	Herpes virus	1 ⁸	Melanoma, stage 3-4	63 years, both	GM-CSF therapy	Deletion of ICP34.5 and ICP47	18	Overall survival	Better
2017, Ma M. S. ⁵⁷	Adenovirus	1 ¹²	Nasopharyngeal carcinoma, stage 2	NA, both	Radiation, cisplatin or 5-fluoro-uracil	TP53	36	Overall survival	Better

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2017, Cohn M.E. 58	Reovirus	3 ¹⁰	Ovarian, tubal, or peritoneal cancer, stage 2-3	60 years, female	Paclitaxel	NA	NA	128	Overall survival	No change
2018, Bradbury P.A. 59	Reovirus	4, 5 ¹²	Lung adenocarcinoma, stage 3-4	64 years, both	Chemotherapy	NA	NA	180	Overall survival	No change
2018, Xiao S.M. 4	Adenovirus	1 ¹²	Advanced unresectable soft-tissue sarcomas, stage 3	49 years, both	Hypothermia alone or in combination with radiotherapy	TP53	NA	NA	Disease control rate	Better
2018, Liu J. 60	Adenovirus	1 ⁹	Hypopharyngeal squamous cell carcinoma	57.9 years, both	Surgery alone or in combination with chemotherapy	TP53	NA	36	Overall survival	Better
2018, NCT01622543	Reovirus	3 ¹⁰	Colorectal cancer, stage 4	50 years, both	Leucovorin, 5-fluorouracil, oxaliplatin or bevacizumab	NA	NA	13	Progression free survival	Worse
2018, NCT01619813	Reovirus	3 ¹⁰	Metastatic prostate adenocarcinoma, stage 4	69 years, male	Docetaxel and Prednisone	NA	NA	20	Progression free survival	Better
2018, NCT01656538	Reovirus	3 ¹⁰	Metastatic breast cancer, stage 4	44 years, female	Paclitaxel	NA	NA	295	Progression free survival	No change
2018, NCT01708993	Reovirus	4, 5 ¹⁰	Non-small cell lung cancer, stage 3-4	63 years, both	Pemetrexed or docetaxel	NA	NA	27	Safety	Safe
2018, NCT01740297	Herpes virus	4 ⁸	Unresectable melanoma, stage 3-4	64.5 years, both	Ipilimumab	GM-CSF	Deletion of ICP34.5 and ICP47	156	Safety	Safe

2018, He C. ⁶¹	Adenovirus	1 ¹²	Hepatocellular carcinoma, stage 1-4	55 years, both	Transarterial chemo-embolization	NA	NA	13	Overall survival	Better
2019, NCT01387555	Vaccinia virus	1 ⁹	Hepatocellular Carcinoma, stage 3	57 years, both	Supportive care	GM-CSF	NA	4.3	Overall survival	No change
2020, Schenk E.L. ⁶²	Picorna-virus	1 ¹¹	Small-cell carcinoma	63 years, both	Saline	NA	Natural	17	Progression free survival	Worse

Note: NA = Not available, GMCSF = Granulocyte macrophage colony-stimulating factor, TP53 = Tumor protein 53, ICP = Infected cell protein

Published articles and clinical trials

Finally, as a reference to design and conduct future systematic analysis on clinical trials, we demonstrate the importance of including multiple databases to retrieve information. Through this study, we found that there were only a few variables showing similarity between the information collected via articles (obtained from PubMed and EMBASE) and trials (from *clinicaltrials.gov*).

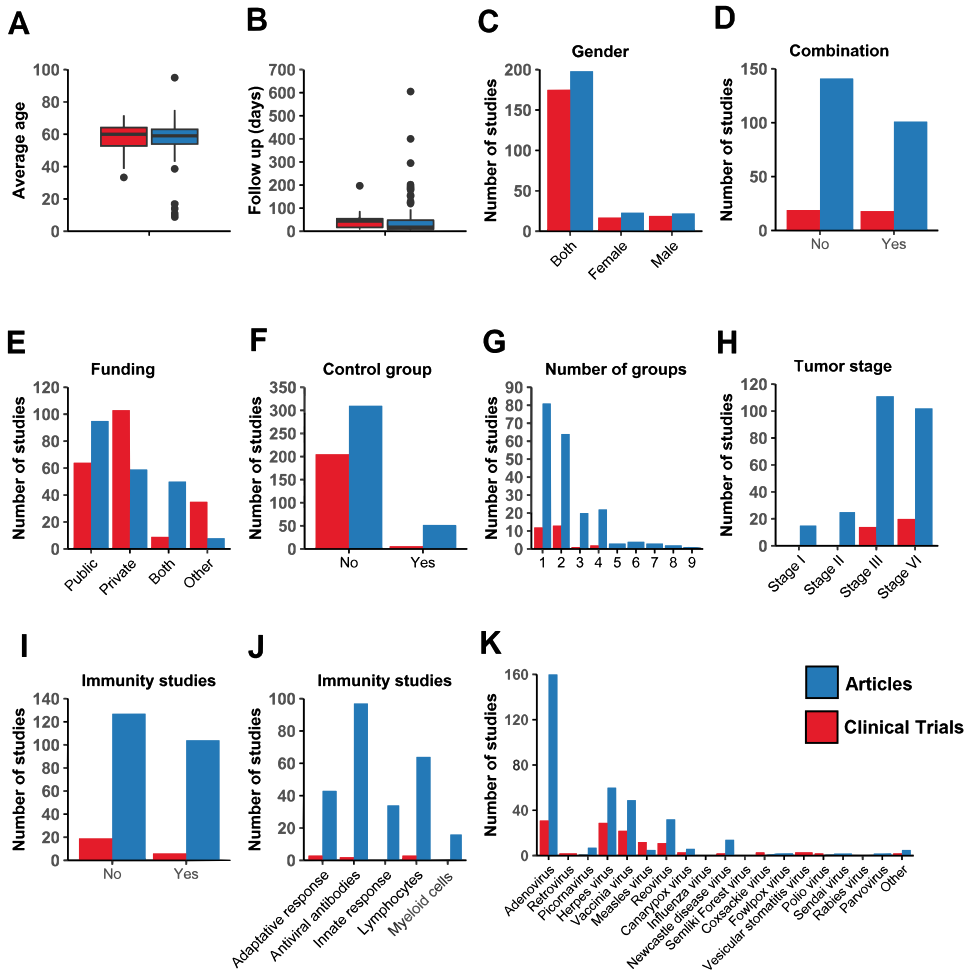


Figure 6: Disparity of data obtained from clinicaltrials.gov and articles in terms of (A) patient age, (B) follow up days, (C) gender, (D) combinatorial strategy with onco-virotherapy, (E) funding, (F) inclusion of control group, (G) number of groups per study, (H) patient tumor-stage, (I) immunological outcomes studied, (J) type of immunological outcomes studied, (K) type of onco-virotherapy studied. Bars in red represent clinical trials and in blue represent articles (retrieved from PubMed and EMBASE).

Patient background-related information, like age (figure 6A), follow up period (figure 6B) and gender (figure 6C), were equal between clinical trial and articles. However, other variables showed high disparity. Information related to trial design, such as type of therapeutic combination were found to be different between the data obtained from trials and articles (figure 6D). Clinical trials were more often funded by private institutions, whilst articles more often received public funding (figure 6E). Furthermore, the number of control groups was lower for the clinical trials compared to the articles (figure 6F), and the number of study groups was smaller (figure 6G). Clinical trials mainly focused on treating patients with advanced and metastatic cancer types, whereas articles also treated stage I and II cancer patients (figure 6H) and investigated immune responses (figure 6I and 6J). Although adenovirus was the most frequently studied viral vector by both clinical trials and articles, clinical trials studied herpes and vaccinia virus almost equally (figure 6K). The described disparity is probably caused by the fact that many trials are not registered on *clinicaltrials.gov*, and that articles on PubMed and EMBASE, in contrast to trials on *clinicaltrials.gov*, are peer-reviewed (figure 1A).

Discussion

Onco-virotherapy is a promising form of immunotherapy for the treatment of cancer. In this review we evaluated the clinical impact of oncovirotherapy for cancer patients that received virotherapy in comparison with cancer patients that received other therapies by means of a systematic analysis. Overall, our results indicate that onco-virotherapy has proven to be safe due to efforts in vector design, rational choices of therapeutic dosage and delivery strategies. Simultaneously, various viral vectors have shown clinical efficacy in terms of better therapeutic outcomes as compared to standard care. Moreover, combinational strategies such as checkpoint blockade, chemotherapy, radiotherapy, and even introduction of immunogenic transgenes has improved clinical efficacy. With this analysis, we aim to provide a reference for clinicians and researchers in the onco-virotherapy field.

Our analysis identified eighteen viral vectors that were used as therapeutic platform to treat twenty-six cancer types. These studies used the following virus-types; adenovirus (42.5% studies), canarypox virus (1.3% studies), coxsackie virus (0.6% studies), fowlpox virus (0.3% studies), herpes virus (21.3% studies), influenza virus (0.1% studies), measles virus (3% studies), Newcastle disease virus (3.6% studies), parvovirus (0.4% studies), picornavirus (1.5% studies), polio virus (0.4% studies), rabies virus (0.1% studies), retrovirus (0.3% studies), reovirus (7.3% studies), Semliki forest virus (0.1% studies), Sendai virus (0.3% studies), vaccinia virus (13.2% studies), and vesicular stomatitis virus (0.9% studies). A wide range of cancers were treated in the clinics such as melanoma (17.1% studies), colon cancer (9.7% studies), lung cancer (7% studies), head and neck cancer (6.8% studies), liver cancer (6.5% studies), ovarian cancer (5.9% studies), pancreatic cancer (5.9% studies), breast cancer (5.6% studies), glioblastoma (5.6% studies), prostate cancer (5.4% studies), sarcoma (4.5% studies), mesothelioma (3.2% studies), bladder cancer (2% studies), glioma (1.8% studies), oesophageal cancer (1.6% studies), renal cancer (1.6% studies), cervical cancer (1.4% studies), cholangiocarcinoma (1.4% studies), rectal cancer (1.4% studies), stomach cancer (1.4% studies), thymus cancer (1.4% studies), thyroid cancer (1.1% studies), diffuse large B cell lymphoma (0.7% studies), acute myeloid leukemia (0.7% studies), adrenocortical cancer (0.5% studies), and uterine cancer (0.2% studies). The clinical studies have been successful in recruiting patient irrespective of gender, age and diversity in tumor types and stages. However, very few studies (<5% studies) have evaluated the potential of onco-virotherapy on paediatric patients, whereas a majority (>80%) of studies were focused on patients of 35 to 70 years in age.

Adenoviruses and herpesviruses were the most utilized virus types in clinical studies. Since 2015, the oncolytic herpes virus T-VEC is globally approved for the treatment of advanced melanoma^{63,64}. Whilst, the genetically modified adenovirus H101 (E1B-55K/E3B-deletion), also known as Oncorine, has been the very first oncolytic virus to be approved in China in 2005 for the treatment of head and neck cancer^{65,66}. Moreover, adenoviruses have been extensively tested as gene therapy vectors, vaccine platforms and synthetic biology tools, so for engineering, adenoviral vectors are more often chosen over other viruses as recently reviewed by Peter and Kuhnel⁶⁷. Similarly, viral vectors with an acceptable safety profile,

such as the vaccinia virus and measles virus, have also been preferred choices for clinical testing^{1,68}. Overall, this suggests that the development and easier accessibility of genetic engineering kits for vector modification has the potential to support the demand for novel viral therapeutics and their assessment in clinical research.

Strikingly, many viruses were not subjected to any genetic modification during the earlier years of onco-virotherapy development, although this approach has the potential to enhance the immunogenicity of viral vectors by the introduction of immunogenic genes. Nonetheless, onco-virotherapy modification gained popularity since 2000, and a large fraction of our analysed trials were initiated in the years thereafter. Considering such native (non-modified) viruses, reovirus has been the most commonly used viral vector in onco-virotherapy that has not undergone genetic modifications⁶⁹. Similarly, canarypox and fowl-pox viruses have been used in their native form to deliver prostate-tumor antigens, as they exhibit a weaker tropism to human cells and preferentially infect tumor cells⁷⁰. Also, vesicular stomatitis virus are being exploited as therapeutic (trial ID: NCT01628640, NCT03120624) due to its sensitivity to interferon-mediated antiviral responses exhibited by normal cells, which allows preferential infection and lysis of tumor cells devoid of active interferon responses⁷¹.

Many of the utilized viral vectors were administered in combination with another form of therapy such as chemotherapy^{35,41,43,44,58,72}, immune checkpoint inhibitors^{14,73,74}, or radiotherapy^{3,4,33,60}. Furthermore, most viruses were injected intratumorally and were administered multiple times, which is not surprising as intratumoral administration of viruses has shown to be effective. Besides, multiple injections increase the possibility of inducing stronger antitumor effects and related immune responses⁷⁵. However, multiple intratumoral injections also face the limitations of an invasive approach, for example in patients with glioblastoma⁷⁶, or in paediatric patients^{77,78}, thus indicating a room for improvement⁷⁹. Additionally, immune responses such as virus neutralization by antiviral antibodies⁸⁰, neutralization mediated by complement activation^{81,82} and cellular-antiviral responses mediated by NK cells⁸³ and T-cell also prove to be a challenge to onco-virotherapy. This has led to an increased requirement of dosage

during subsequent rounds of treatment to counterbalance virus elimination in patients.

In addition to improving the safety and efficacy of viral vectors via genetic modifications and combinatorial approaches, efforts have also been made in overcoming the challenges related to manufacturing a clinical grade stock of these viruses. Factors such as ensuring sterility and proper handling during production, improvement of virus yields, appropriate purification strategies, and formulation for long-term stability and storage have been discussed in detail in existing literature^{84,85}. Moreover, regulatory aspects ranging from virus design and production up to therapeutic utilization in clinics have remained of immense importance for safe application of onco-virotherapy⁸⁶. Taken together, these factors can potentially influence the feasibility of producing the maximum dose required for patient treatment, especially in the case of multiple injections and for virus types that require high dose for effective therapy. Manufacturing challenges can also impact the cost of therapy where the economic evaluation of onco-virotherapy has yet to demonstrate itself as a cheaper alternative to existing therapies^{87,88}.

Non-randomized cohort studies and non-controlled trials have primarily focused on assessing the safety profile of the viral vector and therefore determination of dose limiting toxicity and maximum tolerated dose has remained important. Additionally, side effects such as fever, fatigue, flu-like symptoms, nausea, pain at the site of injection, among others have also been reported to occur post onco-virotherapy, although rarely in severe form⁶.

In most controlled trials, onco-virotherapy treatment resulted in better outcomes for individual variables (>70% of controlled trials) or no change (>40% of controlled trials), although some trials reported worse outcomes (table 1). This indicates that further improvements in onco-virotherapy are still needed. We found that the majority of the studies did not include the immune response in their outcome measures, which was unexpected and remarkable as immunogenic effects are characteristic for onco-virotherapy. Instead, most trials focused on general outcome measures such as progression-free survival, overall survival and tumor size change. Hence, it would be important that in the future onco-virotherapy clinical trials also assess the immune response as an outcome

measure. Moreover, a majority of the trials have chosen clinical criteria of assessment based on the published guidelines such as RECIST⁶. However, recent literature⁸⁹ along with our review indicates the need to establish new parameters to evaluate tumor response to virotherapy in terms of immune-response, reduction of metastasis, and alteration in tumor metabolism and growth.

Only a limited number of trials compared the efficacy of onco-virotherapy with conventional treatments like chemotherapy or radiotherapy (Table 1). For example, the FDA approved therapeutic T-VEC, which is administered intratumorally, has only been studied in comparison with intravenous GM-CSF injections, where T-VEC showed significantly better outcomes⁶⁴. So far the performance of T-VEC in comparison with chemotherapy and/or radiotherapy has not been investigated. Interestingly, recent trials in which T-VEC was combined with checkpoint immunotherapy, resulted in better outcomes as compared to monotherapy of either T-VEC or checkpoint immunotherapy¹⁴. Again, this emphasizes the importance of assessing immune responses post-onco-virotherapy.

From more than 20 different solid tumor types evaluated, skin cutaneous melanoma was most commonly studied. This can likely be ascribed to the fact that this tumor type is accessible for intratumoral injection without the need of surgical interventions. Also, most melanoma cells contain a high mutational burden⁹⁰, and this increases the likelihood of tumor-specific antigen release into the tumor microenvironment upon oncolysis, thereby improving the potency of the onco-virotherapy. Additionally, most tumors studied were in an advanced or metastatic state, likely due to the fact that cancer patients with a good prognosis generally benefit from standard care. This is probably a confounding factor during the assessment of onco-virotherapy, as advanced patients are generally less likely to respond to any therapeutic intervention. Therefore, including patients that suffer from early and localized cancer, and high-risk or early-refractory patients may be a strategy to further explore the effectiveness of onco-virotherapy, as these patients would be more likely to benefit.

Finally, we aspire that the information gathered here can be used as a starting point to construct an interactive database, that provides information to clinicians and researchers who are interested in the therapeutic potential of onco-

virotherapy. Moreover, our search strategies can be used to regularly update such a database, by collecting and screening trial-related data from *clinicaltrials.gov* and articles from PubMed and EMBASE. Further, we encourage clinicians and researchers to continue reviewing literature associated with clinical research by assessing multiple platforms, as it increases the possibilities of finding trial results and articles that are exclusive to a particular platform.

Acknowledgements

Authors' work is supported by CNPq (grant award 305700/2017-0 to R.C.), CAPES (Finance Code 001, to D.B.), and an ATTP-GSMS scholarship (Abel Tasman Talent Program to D.B.). The authors would like to thank Dr. Peter G. Braun and Sjoukje van der Werf from the university library at the University of Groningen for their support in formulation of the search strategy for the systematic review. Moreover, the authors thank the reviewers for their valuable feedback regarding the manuscript.

Ethics statement

Our research did not include any animal experiments or human subjects.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

All authors made substantial contributions to the manuscript. Conception and Design, D.B., L.W., and T.D.; Supervision and Funding Acquisition, T.D. and R.C.; Collection and Assembly of Data, D.B., L.W., and L.R.C.B.; Data Analysis and Interpretation, D.B., L.W., L.R.C.B., T.D., and R.C.; Manuscript Writing, D.B., L.W., L.R.C.B., R.C. and T.D. All authors reviewed the manuscript and approved the final version for submission.

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