Comparative safety and efficacy of oncolytic virotherapy for the treatment of individuals with malignancies: a systematic review, meta-analysis, and Bayesian network meta-analysis

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Summary

Background Oncolytic virotherapy (OV) is an innovative immunotherapy strategy. A comprehensive understanding of oncolytic viruses is essential for advancing research and clinical practice. This analysis aims to evaluate the clinical outcomes of oncolytic virotherapy in cancer patients.

Methods We performed single-arm, pairwise, and Bayesian network meta-analyses, incorporating clinical trials identified through PubMed, Medline, Embase, and the Cochrane Library from database inception to April 30, 2025. Primary endpoints included all-grade and grade ≥3 adverse events (AEs), objective response rate (ORR), and disease control rate (DCR). Effect size measures included risk ratios (RRs) or odds ratios (ORs) with 95% confidence intervals (CIs) or credible intervals (CrIs). Subgroup analyses were conducted to assess outcomes, and meta-regression was applied to evaluate the influence of prognostic variables. This study is registered with PROSPERO, number CRD42022306458.

Findings Of 1976 studies screened, 186 clinical trials with 6979 participants met the inclusion criteria. The most common adverse events associated with oncolytic virotherapy were fatigue (1.98%, 1.71–2.28), pyrexia (2.16%, 1.69–2.69), fever (3.32%, 2.64–4.07), and chills (1.65%, 1.39–1.82), with neutropenia (1.07%, 0.67–1.55) and lymphocytopenia (0.71%, 0.51–0.94) being the predominant severe adverse events. While oncolytic virus monotherapy (OV vs immunotherapy, DCR 2.45, 95% CI 1.60–3.76) and combination regimens (OV plus chemotherapy vs OV, DCR 8.53, 95% CI, 1.97–37.03) enhanced therapeutic efficacy, they presented higher toxicity risks compared to conventional treatments (OV vs immunotherapy, all-grade AE 2.07, 95% CI 1.75–2.44). Notably, combination therapies involving chemotherapy (OV plus chemotherapy vs chemotherapy, all-grade AE 1.10, 95% CI 1.02–1.18) or radiotherapy (OV plus radiotherapy vs radiotherapy, all-grade AE 1.53, 95% CI 1.27–1.84) significantly increase adverse event risks. Conversely, oncolytic virotherapy combined with immunotherapy showed a more favorable safety profile (OV plus immunotherapy vs OV plus chemotherapy, severe AE 0.32, 95% CrI 0.15–0.66) and clinical benefits (OV plus immunotherapy vs OV plus chemotherapy, DCR 0.08, 95% CrI 0.02–0.33). Efficacy varied significantly across treatment strategies (adjusted $p = 0.040$), virus classifications (adjusted $p = 0.0027$), administration routes (adjusted $p = 0.0080$), and patient age groups (adjusted $p = 0.00080$).

Interpretation This analysis provides robust evidence on the tolerability and efficacy of oncolytic virotherapy in cancer treatment. Oncolytic virotherapy demonstrates significant potential as both monotherapy and in combination regimens, offering a favorable balance of efficacy and safety. Virotherapy paired with immunotherapy exhibits a more favorable safety profile, particularly in regimens involving *Reoviridae*- or *Poxviridae*-based strategies. The therapeutic efficacy of oncolytic virotherapy varies notably by multiple factors.

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Research in context

Evidence before this study

Oncolytic virotherapy (OV) is a promising immunotherapy using engineered viruses to target cancer cells and boost anti-tumor immunity. Despite its potential as monotherapy and in combination with other treatments, clinical outcomes vary due to small sample sizes, heterogeneous study designs, and limited exploration of prognostic factors. This study presents a comprehensive meta-analysis to systematically evaluate OV's efficacy, safety, and determinants of therapeutic performance.

Added value of this study

This study synthesizes data from 186 clinical trials with 6979 participants, employing comprehensive meta-analyses to evaluate the safety and efficacy of OV. It highlights superior safety of OV combined with immunotherapy compared to chemotherapy or radiotherapy, while uncovering variations in outcomes based on virus types, administration routes, and patient characteristics.

Implications of all the available evidence

This study highlights the therapeutic potential of OVs in cancer treatment, especially in combination with immunotherapy, offering a promising balance between efficacy and safety. The findings support personalized strategies tailored to patient and disease-specific characteristics to optimize outcomes and minimize risks.

Introduction

Cancer remains a leading global health challenge, with incidence and mortality rates rising significantly worldwide.[1] Recent epidemiological data report 19.3 million new cancer cases and 10.0 million cancer-related deaths globally, marking an increase from 2018 figures of 18.1 million cases and 9.6 million deaths. Strengthening patient management and care is paramount to mitigating this escalating burden. Moreover, there is an urgent need to develop and optimize therapeutic strategies to improve cancer control outcomes.[2–6]

Traditional treatments for advanced cancers, including surgery, chemoradiotherapy, and targeted therapy, provide limited benefits for many patients. In recent years, immunotherapy has emerged as a transformative approach, particularly in the treatment of immunogenic tumors. Immune checkpoint inhibitors, as a cornerstone of immunotherapy, have demonstrated a significant potential by precisely targeting tumor signaling pathways, remodeling the tumor microenvironment, and enhancing antitumor immune responses.[7] Nevertheless, their clinical efficacy is confined to a subset of patients, predominantly due to interindividual variability and substantial tumor heterogeneity. Furthermore, primary or secondary resistance to these therapies remains a significant challenge, underscoring the need for innovative approaches to overcome these barriers.[8]

Oncolytic viruses have been investigated as potential cancer therapies for over a century. With accumulating preclinical evidence highlighting their pivotal role in cancer immunotherapy.[9–12] Clinically, oncolytic viruses are administered via intratumoral injection or systemic delivery, exploiting their tumor-selective replication, oncolytic activity, and capacity for genetic modification to express therapeutic transgenes, such as immune stimulators or antiangiogenic factors.[13,14] Several oncolytic virus platforms, including *herpesviruses*, *adenoviruses*, and *poxviruses*, have advanced to clinical trials, with talimogene laherparepvec (T-VEC) achieving regulatory approval for the treatment of advanced melanoma.

Despite these advances, significant challenges remain in translating oncolytic viruses into widespread clinical application. First, existing meta-analyses on the efficacy of oncolytic virus are confined to specific cancer types (e.g., melanoma, glioblastoma) or focus exclusively on single-arm outcomes, without comparative evaluation against conventional therapies.[15–17] Second, the safety profiles of different virus platforms, particularly DNA-vs RNA-based viruses and combination regimens (e.g., with chemotherapy or checkpoint inhibitors), remain insufficiently quantified due to inconsistent adverse event reporting standards.[13,18] Additionally, key prognostic factors influencing the responses of oncolytic virotherapy, such as viral structure, administration route, and patient demographics, have not been systematically assessed.

While previous meta-analyses have provided valuable insights into the efficacy of oncolytic virotherapy in specific settings, they often focus on single-agent therapies or individual virus platforms, limiting their generalizability.[19,20] Furthermore, heterogeneity in study design, patient populations, and outcome measures has hindered definitive conclusions about the comparative safety and efficacy of different oncolytic virus approaches. To address these gaps, we conducted a comprehensive systematic review, pairwise meta-analysis, and Bayesian network meta-analysis to evaluate the clinical outcomes of oncolytic virotherapy across diverse cancer types and treatment strategies. By integrating a broad spectrum of studies and applying advanced statistical methodologies, this analysis offers

a robust and generalizable assessment of the safety and efficacy of oncolytic virotherapy, providing critical guidance for clinical decision-making and identifying key areas for future research.

Our study aims to summarize and assess the tolerability and efficacy of oncolytic virotherapy using trial-level data and to quantify the impact of key prognostic factors on clinical outcomes, including age, therapeutic regimens, cancer types, virus classifications, virus structure, and administration routes. This comprehensive evaluation seeks to address existing knowledge gaps, establish a benchmark for clinical development, and support the optimization of treatment strategies for cancer patients.

Methods

Search strategy and selection criteria

This meta-analysis was conducted in accordance with PRISMA guidelines.[21,22] This study is registered with PROSPERO, number CRD42022306458 (Appendix p 1). We systematically searched PubMed, Medline, Embase, Cochrane Library, and ClinicalTrials.gov to identify relevant studies published from database inception to April 30, 2025 (Appendix p 2).

Clinical trials were eligible for inclusion if they met the following criteria: (a) Prospective clinical trials published before April 30, 2025, were considered to ensure the inclusion of the most recent and relevant data. (b) Trials investigating any type of malignancy, including both hematological and solid tumors, were included to provide a comprehensive overview of the application of oncolytic virotherapy across different cancer types. (c) Studies involving participants treated with oncolytic virus-based therapies including oncolytic virus-mediated gene delivery, were selected to focus specifically on the therapeutic modality under investigation. (d) Trials that reported outcomes for pediatric participants (<21 years old) or adult participants (≥21 years old) separately, with at least 50% of the cohort in a single age group, were included to facilitate age-specific analyses. (e) Studies providing tabulated data or reporting the overall incidence of treatment-related adverse events were included to enable adequate assessment of safety outcomes. (f) Only studies published in English were included to maintain consistency and ensure accessibility of the data.

Exclusion criteria were as follows: (a) Conference abstracts, which were excluded due to their typically limited data and the potential for duplication with full publications. (b) Studies focusing exclusively on the pharmacodynamics or pharmacokinetics of treatments were excluded, as they lack comprehensive clinical outcomes relevant to the analysis.

The inclusion and exclusion criteria were designed to ensure that the studies included in the meta-analysis were relevant, comprehensive, and provided sufficient data to evaluate the safety and efficacy of oncolytic virotherapy. By adhering to these criteria, the analysis aimed to minimize bias and produce robust findings applicable to a wide range of clinical scenarios. Two investigators (PL and LL) independently conducted the literature search and screened articles for inclusion. All potentially relevant articles were reviewed, regardless of reported outcomes, and discrepancies were resolved through consultation with a third reviewer.

Ethics

No formal ethics approval is required for this review as it involves secondary analysis for previously published studies.

Data analysis

Data extraction was independently performed by LL and HL. Additional information was retrieved from ClinicalTrials.gov as needed. If multiple studies reported on the same cohort, the most detailed and recent study was included.

All statistical analyses were performed using R (version 4.3.0) with the packages meta (version v8.0-1), metafor (version v4.6-0), gemtc (version v1.0-2), and R2jags (version v0.7-1). A p-value of ≤ 0.05 was considered statistically significant, and all tests were two-sided. To address the multiplicity issue arising from multiple clinical endpoints in the data analysis, the Benjamini-Hochberg procedure was applied to adjust p-values, thereby controlling the type I error rate and the false discovery rate while mitigating overly conservative results and preserving statistical power.[23,24] Risk of bias was independently assessed by two investigators (PL and LL) using the Cochrane ROB2 (Risk of Bias 2) tool for randomized parallel-arm trials, the Cochrane ROBINS-I (Risk of Bias in Non-randomized Studies-of Interventions) tool for non-randomized parallel-arm trials, and the MINORS (Methodological Index for Nonrandomized Studies) tool for cohort and single-arm trials (Appendix pp 31–34).[25–28]

The primary outcomes included all-grade adverse events, grade 3 or higher (severe) adverse events (defined according to the Common Terminology Criteria for Adverse Events), objective response rate (defined as the proportion of participants with evaluable anti-tumor activity achieving complete response), and disease control rate (defined as the proportion of participants achieving complete response, partial response, or stable disease). Data were extracted on the number of participants experiencing all-grade and severe adverse events from the safety analysis population of each trial. For tumor response, the number of participants achieving complete response and those with complete response, partial response, or stable disease were collected from the evaluable population. All data were cross-verified by two independent investigators (PL and LL), and any discrepancies were resolved through consultation with the original trial protocols.

Statistics

First, single-arm meta-analyses were conducted using a random-effects model to calculate pooled proportions from eligible studies. Between-study heterogeneity was evaluated using the I^2 statistic.[29,30] Subgroup analyses were performed to investigate critical clinical outcomes and potential modifiers of oncolytic virotherapy tolerability and efficacy. Studies were categorized based on the following key characteristics: (a) Viral species. Differentiating oncolytic viruses such as *Herpesviridae*, *Poxviridae*, *Adenoviridae*. (b) Virus structure. Classifying viruses into DNA or RNA viruses. (c) Delivery routes. Evaluating the effects of intratumoral, intravenous, or intraperitoneal administration. (d) Treatment approaches. Comparing single-agent virotherapy with combination regimens involving chemotherapy or immunotherapy. (e) Cancer types. Assessing outcomes across solid and hematologic malignancies. (f) Age groups. Separating pediatric participants (<21 years) from adult participants (≥21 years).

Second, we conducted pairwise meta-analyses using a random-effects model to account for between-study heterogeneity. Pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were calculated for objective response rate and disease control rate. For safety outcomes, summary risk ratios (RRs) and 95% CIs were derived from the incidence of all-grade and severe adverse events. Heterogeneity across studies was assessed using Cochran's Q test and the I^2 statistic.[26] To explore potential sources of heterogeneity, we performed subgroup analyses as mentioned above. Publication bias, including small-study effects, was evaluated using Egger's regression test of the Intercept.[31,32]

Third, network meta-analyses were performed within a Bayesian framework to integrate direct and indirect evidence from multiple comparisons.[33,34] A random-effects model was employed to account for heterogeneity, which is prevalent in clinical trials. The Bayesian model was implemented using Markov Chain Monte Carlo (MCMC) simulations,[35] with four independent chains running 100,000 inference iterations and a thinning interval of 10 after a burn-in phase of 20,000 iterations.[36] Non-informative priors were used to ensure objectivity in estimating treatment effects, while uniform priors were applied to the heterogeneity parameter to constrain the range of between-study standard deviation (τ).[37,38] Model fit and heterogeneity were assessed using the posterior mean of residual deviance (Dbar) and the deviance information criteria (DIC).[33,39] Pooled estimates were reported as RRs for safety outcomes and ORs for efficacy outcomes, along with their 95% credible intervals (CrIs). The transitivity assumption for indirect comparisons was evaluated using a Bayesian meta-regression model incorporating study-level covariates such as sex distribution, age, sample size, study design, virus classification, delivery route, and cancer type (Appendix p 68 and p 74).[40,41] Local inconsistency was assessed by comparing direct and indirect evidence for specific network nodes, with *p*-values less than 0.1 indicating significant inconsistency.[42,43] Global inconsistency was evaluated by comparing the DIC values of consistency and inconsistency models.[42–44] Node splitting analysis identified inconsistent loops when the inconsistency model exhibited a better fit.[45] The surface under the cumulative ranking curve (SUCRA) and mean ranks were calculated to quantify the relative safety and efficacy of each treatment.[46] Two-dimensional clustered ranking graphs, based on cluster analysis, were generated to identify optimal interventions by considering both efficacy and safety outcomes. To ensure the robustness of our findings, sensitivity analyses were conducted by excluding studies with potential biases or those that deviated from inclusion criteria (e.g., small sample sizes or age groups). The pooled effect sizes from these restricted analyses were compared with those derived from the full dataset. Consistency between the results confirmed the stability and reliability of the conclusions, while significant discrepancies suggested the need for further investigation into potential biases. These rigorous analytical approaches provide a comprehensive assessment of the clinical outcomes of oncolytic virotherapy, addressing heterogeneity and bias while informing evidence-based treatment strategies.

Role of funding source

There was no funding source for this study.

Results

A total of 1976 studies were identified from the electronic databases (Fig. 1). After duplicate removal and full-text screening, 247 potentially relevant studies were shortlisted, of which 186 studies (encompassing 6979 participants) met the eligibility criteria. These included 155 single arm trials (3573 participants) and 31 parallel-arm trials (3406 participants). The age of participants ranged from 3 to 97 years. The 186 eligible trials were published between 2001 and 2025, though studies utilizing oncolytic viruses for cancer treatment data back as early as 1998 (Appendix p 30). Most trials were conducted in Europe and the United States, with some involving multicenter collaborations across various countries.

Among the eligible studies, 185 trials (6779 participants) reported the incidence of adverse events (all-grade and/or grade ≥3 adverse events), and 153 trials (5949 participants) were included for response evaluation. Of these, 174 trials focused exclusively on adult patients, while 12 included pediatric populations. Notably, five studies included both adult and pediatric participants, with one study reporting separate data for these groups.

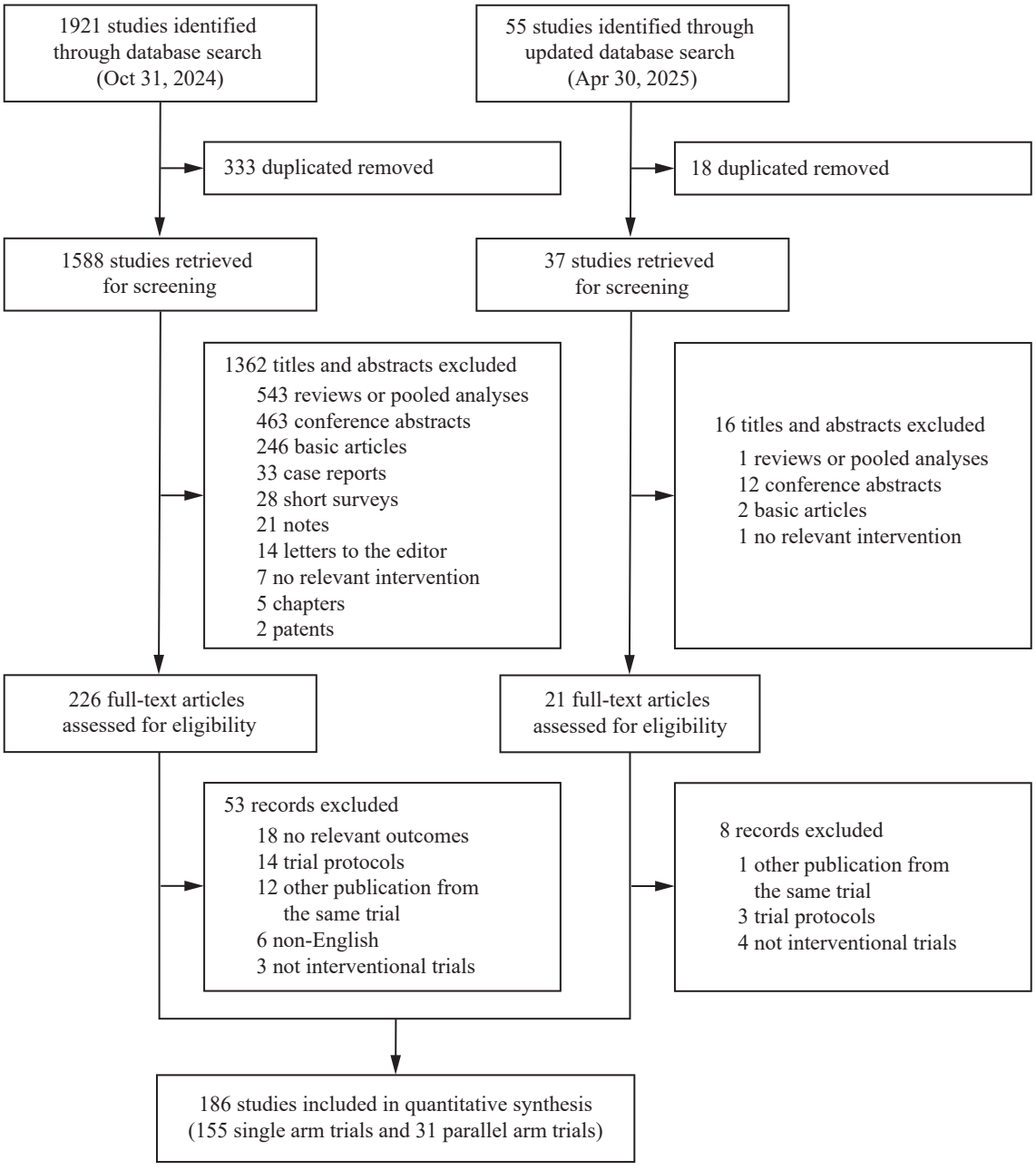

Fig. 1: **Selection of included trials**.

In total, 89 trials involving 2395 participants evaluated the safety and efficacy profiles of oncolytic virus monotherapy. The investigated virus included *vaccinia virus, reovirus, adenovirus, herpes simplex virus, Seneca Vally virus, Newcastle disease virus, hemagglutinating virus, parvovirus, measles virus, vesicular stomatitis virus,* and *poliovirus*. Furthermore, 97 trials (4584 participants) comparing adverse events and/or clinical responses between combination treatments and oncolytic virus monotherapy. These combination regimens often incorporated chemotherapy, radiotherapy, immunotherapy, or targeted therapy and involved *herpes simplex virus, reovirus, adenovirus, vaccinia virus, measles virus, coxsackievirus A21, Seneca Vally virus, fowlpox virus*.

The trials addressed wide range of cancer types, including prostate cancer (n = 9), lung cancer (n = 5), malignant gynecologic disease (n = 9), breast cancer (n = 7), esophageal cancer (n = 1), colorectal cancer

(n = 6), pancreatic cancer (n = 12), liver cancer (n = 11), melanoma (n = 33), epithelial cancer (n = 2), hematologic malignant neoplasm (n = 4), bladder cancer (n = 5), sarcoma (n = 7), squamous cell carcinoma (n = 5), basal cell carcinoma (n = 1), malignant glioma (n = 20), peritoneal carcinomatosis (n = 1), mesothelioma (n = 4), and mixed cancer types (n = 44). Detailed listings of the eligible studies are provided in Appendix pp 3–17 and the summary of characteristics of the trials are displayed in Appendix pp 18–29.

Safety and tolerability

In the single-arm meta-analysis, the overall incidence of all-grade adverse events associated with oncolytic virotherapy was 26.60% (24.39–28.88, I^2 = 98.70%; Appendix pp 36–46). Across the included studies, over 500 different types of adverse events were reported, with several clinically relevant events commonly observed in practice. As detailed in Appendix p 35, the most frequently reported all-grade adverse events included fatigue (1.98%, 1.71–2.28), nausea (1.54%, 1.30–1.78), fever (3.32%, 2.64–4.07), chills (1.65%, 1.39–1.82), diarrhea (1.09%, 0.90–1.30), pyrexia (2.16%, 1.69–2.69), vomiting (1.01%, 0.85–1.18), anemia (1.53%, 1.20–1.90), headache (1.00%, 0.81–1.20), and transaminitis (1.34%, 1.05–1.66). Prespecified subgroup analyses revealed significant differences between treatment strategies, including oncolytic virus monotherapy, virotherapy combined with immunotherapy, multiple immunotherapies, chemotherapy, radiotherapy, chemoradiotherapy, immunoradiotherapy, immunochemotherapy, or standard therapy (p < 0.00010, adjusted p = 0.00050; Appendix pp 47–50). The highest proportion of all-grade adverse events occurred in virotherapy combined with chemoradiotherapy (42.05%, 24.01–61.26), whereas the lowest was observed in virotherapy combined with immunoradiotherapy (10.50%, 7.90–13.43). Subgroup analyses based on virus-derived classifications also identified significant differences (p < 0.00010, adjusted p = 0.00050), with the highest incidence observed in *hemagglutinating viruses* (41.87%, 17.70–68.30) and the lowest in *parvoviruses* (13.43%, 1.38–32.10). Furthermore, the incidence of all-grade adverse events demonstrated a significant association with sex (p = 0.0020, adjusted p = 0.0080), with females exhibiting a slightly higher incidence (38.26%, 30.33–46.52) compared to males (35.47%, 24.51–47.26). No statistically significant differences were identified in the incidence of all-grade adverse events based on virus-derived structures (p = 0.38, adjusted p = 0.61), delivery routes (p = 0.13, adjusted p = 0.17), cancer types (p = 0.28, adjusted p = 0.45), or age (p = 0.72, adjusted p = 0.72). These findings suggest that the incidence of all-grade adverse events was closely related to the type of viral species employed in oncolytic virotherapy.

The pooled incidence of grade 3 or higher (severe) adverse events was 2.12% (1.73–2.55, I^2 = 93.50%; Appendix pp 36–46). As detailed in Appendix p 35, the most frequently reported severe adverse events included neutropenia (1.07%, 0.67–1.55), anemia (0.42%, 0.25–0.62), lymphocytopenia (0.71%, 0.51–0.94), fatigue (0.31%, 0.20–0.45), leukopenia (0.66%, 0.38–1.00), transaminitis (0.34%, 0.20–0.50), fever (0.18%, 0.08–0.31), thrombocytopenia (0.42%, 0.24–0.65), diarrhea (0.16%, 0.09–0.26), and dyspnea (0.18%, 0.09–0.29). Subgroup analyses revealed significant associations between the incidence of severe adverse events and oncolytic virus-based treatment strategies (p = 0.00040, adjusted p = 0.00053), virus-derived classifications (p < 0.00010, adjusted p = 0.00050), and delivery routes (p = 0.0040, adjusted p = 0.0080; Appendix pp 47–50). Among virus-derived classifications, *vesicular stomatitis virus* demonstrated the highest incidence of severe adverse events (5.40%, 1.67–10.99), whereas *herpes simplex virus* exhibited the lowest (1.35%, 0.93–1.85). RNA-derived oncolytic viruses had a higher incidence of severe adverse events (2.87%, 1.91–3.99) compared to DNA-derived viruses (1.82%, 1.44–2.25). Regarding delivery routes, intravenous administration (3.28%, 2.36–4.32) was associated with a higher incidence of severe adverse events than intratumoral injection (1.70%, 1.28–2.16), intraperitoneal injection (1.33%, 0.33–2.87), or multiple delivery routes (2.06%, 1.19–3.12). Combination regimens exhibited a higher incidence of severe adverse events (2.74%, 2.12–3.44) compared to oncolytic virus monotherapy (1.54%, 1.08–2.06; p = 0.014, adjusted p = 0.028). Among combination strategies, virotherapy combined with chemoradiotherapy had the highest incidence of severe adverse events (5.79%, 1.21–13.24), whereas virotherapy combined with immunotherapy had the lowest (1.18%, 0.76–1.66).

Pairwise meta-analyses were conducted to evaluate various treatment strategies, including oncolytic virus monotherapy vs immunotherapy, virotherapy combined with immunotherapy vs immunotherapy, virotherapy combined with immunotherapy vs oncolytic virus monotherapy, virotherapy combined with chemotherapy vs chemotherapy, virotherapy combined with chemotherapy vs oncolytic virus monotherapy, and virotherapy combined with standard therapy vs standard therapy (Fig. 2). A comprehensive summary of the pairwise comparisons is presented in Appendix pp 54–55. In terms of safety outcomes, virotherapy combined with chemotherapy or virotherapy combined with radiotherapy was significantly associated with an increased incidence of all-grade adverse events compared to chemotherapy (RR 1.10, 95% CI 1.02–1.18, I^2 = 64.60%) or radiotherapy (RR 1.53, 95% CI 1.27–1.84), respectively (Appendix pp 56–58). Similarly, virotherapy combined with immunotherapy was associated with higher risks of both all-grade adverse events (RR 1.30, 95% CI 1.15–1.48, I^2 = 67.50%) and severe adverse events (RR 1.46, 95% CI 1.14–1.88, I^2 = 0.00%)

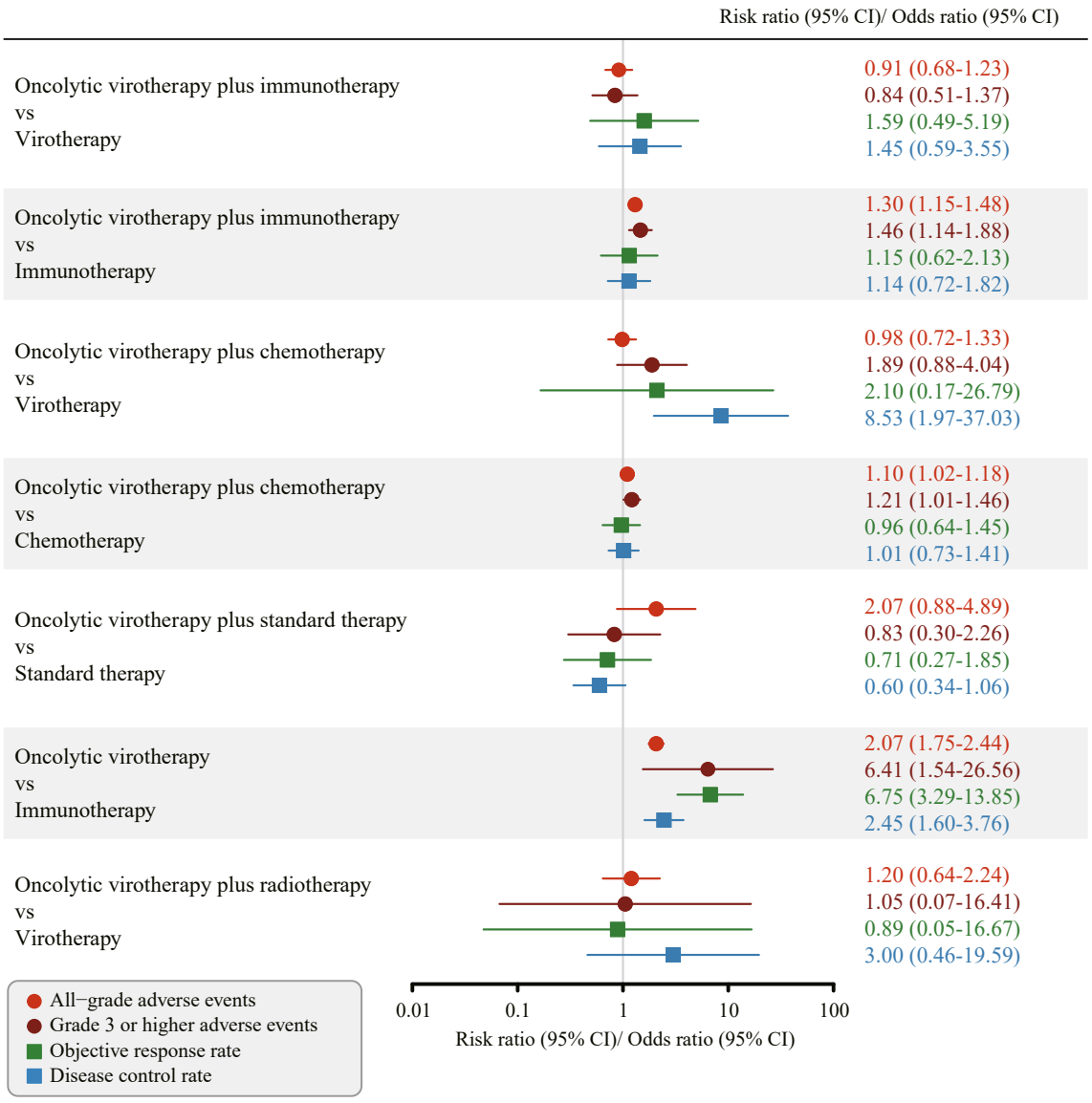

***Fig. 2:* Pooled toxic effects and efficacies of head-to-head comparisons of treatment strategies by pairwise meta-analysis**. Pooled risk ratios (RRs) with corresponding 95% confidence intervals (CIs) for toxic effects including all-grade adverse events (red dots and horizontal lines) and grade 3 or higher adverse events (brown dots and horizontal lines). Pooled odds ratios (ORs) with corresponding 95% confidence intervals (CIs) for efficacies including objective response rate (green squares and horizontal lines) and disease control rate (blue squares and horizontal lines). Dots represent study-specific RRs and squares represent study-specific ORs. Horizontal lines indicate the 95% CIs.

compared with immunotherapy alone. Moreover, oncolytic virus monotherapy was significantly less well tolerated than immunotherapy (cytokine-based), demonstrating higher risks for both all-grade adverse events (RR 2.07, 95% CI 1.75–2.44) and severe adverse events (RR 6.41, 95% CI 1.54–26.56). An assessment of publication bias using Egger's test revealed no significant publication bias for either all-grade adverse events (p = 0.65, adjusted p = 0.99) or severe adverse events (p = 0.99, adjusted p = 0.99; Appendix p 55).

In the network meta-analysis, 32 head-to-head trials were included for detailed evaluation of eight treatment strategies in oncolytic virotherapy: oncolytic virus monotherapy, virotherapy combined with immunotherapy, virotherapy combined with multiple immunotherapies, virotherapy combined with chemotherapy, virotherapy combined with radiotherapy, immunotherapy, chemotherapy, and radiotherapy. All treatment strategies were evaluated for both all-grade adverse events and severe adverse events. The overall results of

the tolerability outcomes are presented in Appendix pp 62–69. Model fit and global consistency were assessed using the DIC. A consistency random-effects model was employed to analyze all-grade adverse events (DIC = 124.20, I^2 = 9.00%) and severe adverse events (DIC = 107.82, I^2 = 0.00%; Appendix p 64). For the safety profiles, oncolytic virus monotherapy (RR 1.69, 95% CrI 1.32–2.13), virotherapy combined with immunotherapy (RR 1.37, 95% CrI 1.12–1.64), virotherapy combined with multiple immunotherapies (RR 1.85, 95% CrI 1.27–2.70), virotherapy combined with chemotherapy (RR 1.55, 95% CrI 1.14–2.09), and chemotherapy (RR 1.43, 95% CrI 1.02–2.00) were associated with higher risks of all-grade adverse events compared to immunotherapy alone. Additionally, oncolytic virus monotherapy posed a higher risk than virotherapy combined with immunotherapy (RR 1.23, 95% CrI 1.04–1.52). Furthermore, virotherapy combined with radiotherapy resulted in a significantly higher risk of all-grade adverse events compared to radiotherapy alone (RR 1.53, 95% CrI 1.02–2.34; Appendix p 64).

In terms of severe adverse events, the analysis revealed that oncolytic virus monotherapy (RR 2.63, 95% CrI 1.41–5.56), virotherapy combined with immunotherapy (RR 1.64, 95% CrI 1.11–2.56), virotherapy combined with chemotherapy (RR 5.26, 95% CrI 2.44–12.50), and chemotherapy alone (RR 4.24, 95% CrI 1.88–10.46) were associated with a higher incidence of severe adverse events compared to immunotherapy. Additionally, virotherapy combined with chemotherapy (RR 3.13, 95% CrI 1.51–6.73) and chemotherapy alone (RR 2.58, 95% CrI 1.19–5.81) demonstrated lower tolerability compared to virotherapy combined with multiple immunotherapies (Appendix p 64). Bayesian clustered ranking based on SUCRA values for toxicity revealed that virotherapy combined with radiotherapy (SUCRA 80.80%) was most likely to rank highest for all-grade adverse events, while virotherapy combined with chemotherapy (SUCRA 89.20%) ranked highest for severe adverse events (Appendix pp 68–69). Conversely, immunotherapy was identified as the least toxic treatment, with SUCRA values of 4.60% and 9.80% for all-grade adverse events and severe adverse events, respectively. Model consistency was evaluated using node-splitting analysis, which showed no statistically significant discrepancies between direct and indirect estimates (Appendix p 67). Meta-regression analysis further indicated that sample size and participant age significantly influenced the effect sizes for both all-grade and severe adverse events (Appendix p 68). Consequently, sensitivity analyses were performed by excluding studies with sample size below 15 and those involving pediatric patients. These preplanned sensitivity analyses confirmed the robustness of the primary findings, with no significant deviations observed (Appendix pp 76–83).

In addition, we investigated the impact of oncolytic virus classification on clinical outcomes by analyzing data from 33 head-to-head trials (Appendix pp 70–75). The oncolytic viruses evaluated in these studies were categorized into five families: *Herpesviridae*, *Adenoviridae*, *Picornaviridae*, *Reoviridae*, and *Poxviridae*. Ten treatment modalities were identified: *Herpesviridae* monotherapy, *Herpesviridae*-based combination therapy, *Adenoviridae* monotherapy, *Adenoviridae*-based combination therapy, *Picornaviridae* monotherapy, *Picornaviridae*-based combination therapy, *Reoviridae*-based combination therapy, *Poxviridae*-based combination therapy, *Poxviridae*-based multimodal therapy, and standard therapy. A consistency random-effects model was employed to evaluate all-grade adverse events (DIC = 128.16, I^2 = 6.00%) and severe adverse events (DIC = 112.67, I^2 = 0.00%; Appendix p 72). As shown in Fig. 3A, *Herpesviridae* monotherapy was associated with significantly higher risks of both all-grade adverse events (RR 1.89, 95% CrI 1.35–2.63) and severe adverse events (RR 2.44, 95% CrI 1.11–6.25) compared to standard therapy. Furthermore, *Herpesviridae*-based combination therapy demonstrated an elevated risk of all-grade adverse events relative to *Reoviridae*-based combination therapy (RR 1.58, 95% CrI 1.13–2.31), *Poxviridae*-based combination therapy (RR 1.58, 95% CrI 1.06–2.51), and standard therapy (RR 1.72, 95% CrI 1.33–2.27). *Picornaviridae*-based combination therapy was liked to a higher risk of severe adverse events compared to *Picornaviridae* monotherapy (RR 2.93, 95% CrI 1.01–8.15). Notably, the results suggested that *Reoviridae*-based combination therapy (RR 0.22, 95% CrI 0.14–0.86) and *Poxviridae*-based combination therapy (RR 0.21, 95% CrI 0.14–0.85) were less toxic in terms of severe adverse events vs *Herpesviridae* monotherapy. Treatment rankings based on SUCRA values indicated that standard therapy ranked highest for both all-grade adverse events (SUCRA 15.70%) and severe adverse events (SUCRA 23.30%; Fig. 4A and Appendix p 75). In contrast, *Herpesviridae*-based combination therapy (SUCRA 86.10%) ranked as the worst for all-grade adverse events, while *Herpesviridae* monotherapy (SUCRA 90.90%) ranked highest for severe adverse events. The model's local inconsistency was assessed using node-splitting analyses, which revealed no significant differences between direct and indirect estimates (Appendix p 73). Furthermore, preplanned sensitivity analyses confirmed the robustness of the primary findings (Appendix pp 84–91).

Based on the analyses, the most commonly reported adverse events associated with oncolytic virotherapy were fatigue, pyrexia, fever, and chills, while the most prevalent severe adverse events included neutropenia and lymphocytopenia. Although both monotherapy and combination regimens involving oncolytic viruses may enhance therapeutic efficacy, they are associated with increased toxicity risks compared to conventional

A

All-grade adverse events | Treatment | Grade 3 or higher adverse events

Herpesviridae (monotherapy)	0.63 (0.29-1.30)	0.23 (0.03-1.20)	0.23 (0.04-1.05)	0.26 (0.02-2.15)	0.75 (0.06-7.19)	**0.22 (0.14-0.86)**	**0.21 (0.14-0.85)**	0.24 (0.03-1.38)	**0.41 (0.16-0.90)**
1.09 (0.83-1.43)	**Herpesviridae (combination therapy)**	0.88 (0.26-2.76)	0.89 (0.33-2.31)	1.01 (0.09-5.28)	2.90 (0.35-9.73)	0.87 (0.42-1.67)	0.80 (0.32-1.75)	0.95 (0.25-3.37)	0.64 (0.34-1.10)
1.09 (0.59-1.97)	1.17 (0.70-2.05)	**Adenoviridae (monotherapy)**	1.02 (0.53-1.98)	1.17 (0.09-4.99)	3.40 (0.34-7.37)	0.99 (0.33-2.87)	0.91 (0.28-2.89)	1.09 (0.23-4.91)	0.83 (0.30-2.26)
1.32 (0.75-2.3)	1.42 (0.90-2.35)	1.22 (0.97-1.52)	**Adenoviridae (combination therapy)**	1.14 (0.10-4.29)	3.29 (0.38-10.4)	0.97 (0.41-2.23)	0.89 (0.33-2.32)	1.07 (0.26-4.16)	0.81 (0.37-1.75)
1.58 (0.53-4.23)	1.71 (0.60-4.48)	1.45 (0.49-3.94)	1.20 (0.41-3.15)	**Picornaviridae (monotherapy)**	**2.93 (1.01-8.15)**	0.86 (0.02-8.78)	0.78 (0.02-8.41)	0.93 (0.12-12.14)	0.72 (0.02-7.07)
1.51 (0.53-3.76)	1.63 (0.61-3.93)	1.39 (0.49-3.52)	1.14 (0.41-2.80)	0.95 (0.67-1.36)	**Picornaviridae (combination therapy)**	0.30 (0.01-2.33)	0.27 (0.01-2.24)	0.32 (0.21-3.46)	0.25 (0.01-1.88)
1.47 (0.93-2.29)	**1.58 (1.13-2.31)**	1.36 (0.83-2.19)	1.12 (0.72-1.72)	0.93 (0.37-2.58)	0.97 (0.42-2.58)	**Reoviridae (combination therapy)**	0.92 (0.45-1.85)	1.10 (0.32-3.61)	0.84 (0.58-1.22)
1.47 (0.89-2.48)	**1.58 (1.06-2.51)**	1.35 (0.80-2.35)	1.11 (0.69-1.84)	0.93 (0.36-2.71)	0.98 (0.40-2.67)	1.00 (0.70-1.47)	**Poxviridae (combination therapy)**	1.19 (0.45-3.14)	0.91 (0.51-1.69)
1.17 (0.56-2.41)	1.26 (0.66-2.53)	1.08 (0.51-2.27)	0.89 (0.44-1.82)	0.74 (0.25-2.36)	0.78 (0.28-2.38)	0.79 (0.42-1.51)	0.80 (0.47-1.33)	**Poxviridae (multimodal therapy)**	0.77 (0.24-2.40)
1.89 (1.35-2.63)	**1.72 (1.33-2.27)**	1.54 (0.99-2.37)	1.27 (0.87-1.85)	1.06 (0.43-2.90)	1.11 (0.49-2.85)	1.14 (0.93-1.40)	1.14 (0.82-1.54)	1.43 (0.78-2.61)	**Standard therapy**

B

Disease control rate | Treatment | Objective response rate

Herpesviridae (monotherapy)	1.40 (0.40-7.10)	**0.02 (0.01-0.45)**	0.07 (0.01-1.11)	0.33 (0.12-6.41)	0.23 (0.12-3.42)	0.26 (0.17-1.46)	0.23 (0.14-2.24)	0.16 (0.07-4.06)	0.59 (0.17-3.10)
0.53 (0.18-1.30)	**Herpesviridae (combination therapy)**	**0.05 (0.02-0.68)**	0.15 (0.04-1.59)	0.71 (0.24-10.03)	0.51 (0.15-4.97)	0.58 (0.18-1.66)	0.52 (0.28-2.85)	0.35 (0.12-5.96)	0.43 (0.14-1.20)
8.70 (0.15-20.23)	8.45 (0.17-15.92)	**Adenoviridae (monotherapy)**	3.30 (0.86-13.28)	15.43 (0.34-26.32)	11.21 (0.37-23.91)	12.73 (0.86-19.52)	11.34 (0.54-23.31)	7.61 (0.56-13.24)	12.22 (0.92-17.34)
0.77 (0.22-4.43)	0.75 (0.22-3.97)	**0.09 (0.03-0.24)**	**Adenoviridae (combination therapy)**	4.63 (0.14-13.05)	3.28 (0.15-8.73)	3.74 (0.37-11.35)	3.35 (0.23-9.64)	2.26 (0.56-10.38)	3.64 (0.40-13.10)
0.63 (0.27-4.58)	0.59 (0.28-4.33)	0.07 (0.02-5.18)	0.80 (0.21-5.41)	**Picornaviridae (monotherapy)**	0.71 (0.18-3.27)	0.81 (0.36-13.72)	0.73 (0.24-15.34)	0.50 (0.21-8.42)	0.79 (0.16-11.61)
0.74 (0.18-4.03)	0.69 (0.13-3.75)	0.08 (0.04-5.40)	0.94 (0.21-5.59)	1.12 (0.34-3.44)	**Picornaviridae (combination therapy)**	1.13 (0.12-11.51)	1.01 (0.58-14.18)	0.69 (0.12-8.89)	1.09 (0.13-9.84)
1.57 (0.44-3.98)	1.44 (0.63-3.47)	0.17 (0.06-8.86)	1.97 (0.14-8.39)	1.08 (0.15-6.94)	0.97 (0.21-4.42)	**Reoviridae (combination therapy)**	0.88 (0.17-4.92)	0.60 (0.23-13.90)	0.97 (0.48-2.01)
2.74 (0.81-9.31)	**2.47 (1.05-9.29)**	0.31 (0.11-7.31)	3.54 (0.57-13.05)	4.33 (0.63-8.41)	1.95 (0.38-12.39)	2.00 (0.63-8.11)	**Poxviridae (combination therapy)**	0.69 (0.26-6.80)	1.09 (0.23-5.01)
1.01 (0.50-9.58)	0.97 (0.11-9.59)	0.11 (0.04-3.26)	1.29 (0.32-14.23)	1.64 (0.18-15.97)	0.75 (0.05-10.69)	0.77 (0.07-8.65)	0.38 (0.15-2.39)	**Poxviridae (multimodal therapy)**	1.61 (0.19-4.11)
0.91 (0.27-2.33)	1.67 (0.77-3.85)	0.17 (0.06-8.24)	1.93 (0.34-13.30)	1.07 (0.17-5.93)	0.95 (0.24-3.61)	0.98 (0.48-1.96)	0.57 (0.20-1.15)	1.48 (0.27-12.11)	**Standard therapy**

Fig. 3: **Multiple comparisons for toxic effects and efficacies in network meta-analysis.** (A) Data are risk ratios (RRs) and corresponding 95% credible intervals (CrIs) in the column-defining treatment is compared to row-defining treatment. For all-grade adverse events in the left lower half and grade 3 or higher adverse events in the right upper half, a risk ratio (RR) lower than 1 favors the column-defining treatment. Cells in bold print indicate significant results. (B) Data are odds ratios (ORs) and corresponding 95% credible intervals (CrIs) in the column-defining treatment is compared to row-defining treatment. For disease control rate in the left lower half and objective response rate in the right upper half, an odds ratio (OR) below 1 favors the row-defining treatment. Significant results are in bold.

treatments such as standard chemotherapy or radiotherapy. Notably, combination therapies that incorporate chemotherapy or radiotherapy significantly heightened the risks of both adverse events and severe adverse events compared to oncolytic virus monotherapy. Conversely, oncolytic virotherapy combined with immunotherapy demonstrated a more favorable safety profile, characterized by lower adverse event rates and corroborated by observed clinical benefits. In particular, regimens combining multiple immunotherapies or employing *Reoviridae*- or *Poxviridae*-based strategies showed superior control of toxicity. These findings highlight the necessity of rigorous benefit-risk assessments when integrating these therapies into

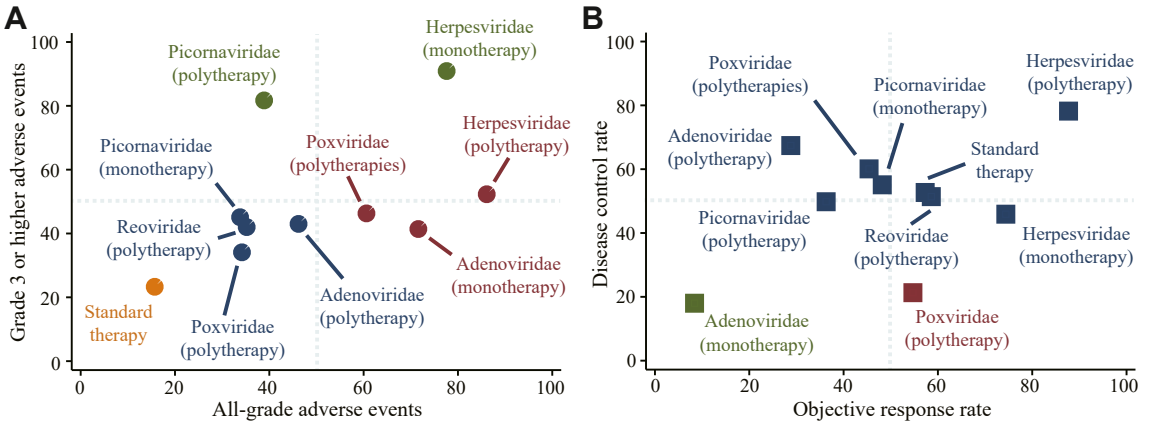

Fig. 4: **Two-dimensional ranking plots of treatment strategies for overall safety (A) and benefit (B).** (A) For all-grade adverse events and grade 3 or higher adverse events, treatments lying in the lower left are less toxic. (B) For objective response rate and disease control rate, treatments lying in the upper right are more effective.

clinical practice, underscoring the importance of tailoring treatment strategies to individual patient needs.

Efficacy

In the single-arm meta-analysis, the pooled objective response rate and disease control rate across all oncolytic virus-based treatment regimens were 13.15% (10.29–16.24, I^2 = 85.60%) and 53.96% (49.47–58.42, I^2 = 85.30%), respectively (Appendix pp 36–46). Subgroup analyses revealed significant associations between objective response rate and factors such as virus-based treatments (p = 0.040, adjusted p = 0.040), virus-derived classifications (p = 0.0020, adjusted p = 0.0027), delivery routes (p = 0.0040, adjusted p = 0.0080), and patient age (p = 0.00020, adjusted p = 0.00080; Appendix pp 50–53). Among virus classifications, *poliovirus* exhibited the most favorable objective response rate (25.00%, 3.93–53.92), whereas *measles virus* demonstrated the highest disease control rate (80.14%, 52.54–98.07). Combination virotherapy strategies achieved a higher objective response rate (18.01%, 13.68–22.71, I^2 = 86.80%) compared to monotherapy (8.42%, 5.12–12.27, I^2 = 82.80%; p = 0.0020, adjusted p = 0.0076). Intratumoral administration yielded the highest objective response rate (16.78%, 12.58–21.37, I^2 = 87.80%) compared to intravenous (8.57%, 5.36–12.29, I^2 = 70.30%) and intraperitoneal injections (7.11%, 0.07–22.21, I^2 = 84.90%). Adults demonstrated higher objective response rate (14.13%, 11.07–17.44, I^2 = 86.10%) than children (0.50%, 0.04–5.16, I^2 = 7.80%; p = 0.00020, adjusted p = 0.00080).

The pairwise comparison meta-analysis suggested that oncolytic virus monotherapy was more effective than cytokine-based immunotherapy in terms of objective response rate (OR 6.75, 95% CI 3.29–13.85) and disease control rate (OR 2.45, 95% CI 1.60–3.76; Appendix pp 54–55). Additionally, oncolytic virotherapy combined with chemotherapy significantly improved disease control rate compared to oncolytic virus monotherapy (OR 8.53, 95% CI 1.97–37.03, I^2 = 42.80%). Subgroup analysis by virus-derived classifications identified adenovirus-based combination therapies as significantly associated with enhanced disease control rate (OR 6.05, 95% CI 1.35–27.17, I^2 = 46.40%; Appendix pp 59–61). No publication bias was detected for objective response rate (p = 0.75, adjusted p = 0.99) and disease control rate (p = 0.43, adjusted p = 0.99), as assessed by Egger's test (Appendix p 55).

In the network meta-analysis, seven therapeutic strategies were evaluated for objective response rate and disease control rate: oncolytic virus monotherapy, virotherapy combined with immunotherapy, virotherapy combined with multiple immunotherapies, virotherapy combined with chemotherapy, virotherapy combined with radiotherapy, immunotherapy, and chemotherapy. A consistency random-effects model was employed to assess the objective response rate (DIC = 100.05, I^2 = 0.0%) and disease control rate (DIC = 113.02, I^2 = 10.0%; Appendix p 66). The comprehensive results for efficacy outcomes are detailed in Appendix pp 62–69. Virotherapy combined with chemotherapy demonstrated superior efficacy compared to immunotherapy in terms of disease control rate (OR 2.34, 95% CrI 1.41–8.74). Additionally, virotherapy plus chemotherapy (OR 6.67, 95% CrI 1.14–33.33; OR 10.78, 95% CrI 1.18–29.84) and chemotherapy alone (OR 6.67, 95% CrI 1.11–50.00; OR 11.71, 95% CrI 1.20–25.76) outperformed virotherapy combined with multiple immunotherapies in both objective response rate and disease control rate (Appendix p 65). Treatment rankings based on SUCRA values indicated that immunotherapy was the least effective in achieving disease control (SUCRA 12.80%;

Appendix pp 68 and 69). Conversely, virotherapy combined with chemotherapy and chemotherapy alone ranked highest for both objective response rate (SUCRA 79.7%; 83.80%) and disease control rate (SUCRA 87.30%; 90.10%). Statistical test revealed no significant inconsistency between direct and indirect estimates (Appendix p 67). Furthermore, sensitivity analyses confirmed that the results of the network meta-analysis remained consistent with the original findings (Appendix pp 76–83).

When evaluating the efficacy of oncolytic virus classifications, *Adenoviridae* monotherapy was found to be less effective than *Herpesviridae* monotherapy (OR 0.02, 95% CrI 0.01–0.45) and *Herpesviridae*-based combination therapy (OR 0.05, 95% CrI 0.02–0.68) in terms of objective response rate (Fig. 3B and Appendix pp 70–75). Moreover, *Herpesviridae*-based combination therapy demonstrated superior disease control compared to *Poxviridae*-based combination therapy (OR 2.47, 95% CrI 1.05–9.29). SUCRA rankings identified *Adenoviridae* monotherapy as the least effective treatment modality for both objective response rate (SUCRA 8.40%) and disease control rate (SUCRA 18.00%; Fig. 4B and Appendix p 75). In contrast, *Herpesviridae*-based combination therapy was most likely to achieve the best outcomes for objective response rate (SUCRA 87.70%) and disease control rate (SUCRA 78.20%). To ensure robustness, the consistency random-effects model was used to analyze objective response rate (DIC = 104.52, I^2 = 0.00%) and disease control rate (DIC = 121.10, I^2 = 8.00%; appendix p 72). Node-splitting analysis confirmed no significant local inconsistencies between direct and indirect estimates (Appendix p 73). The analysis showed no statistical differences between the direct and indirect estimates. Sensitivity analyses reaffirmed that the main findings were unaffected by preplanned methodological adjustments (Appendix pp 84–91).

In summary, the therapeutic efficacy of oncolytic virotherapy varies substantially depending on treatment strategies, viral classifications, administration routes, and patient demographics. Oncolytic virus monotherapy superior performance compared to immunotherapy in specific efficacy parameters, while the addition of chemotherapy further enhances disease control rates. Combination therapies, particularly those utilizing *herpes simplex virus*, achieve higher objective response rates and disease control rates but are associated with an increased risk of toxicity. *Adenovirus*-based combination regimens also show potential in improving disease control rates. Among administration routes, intratumoral injection appear to offer superior therapeutic efficacy. Notably, adults exhibit a more favorable response to oncolytic virotherapy than pediatric patients. For individuals unresponsive to immunotherapy, oncolytic virus monotherapy may represent a viable alternative. These findings underscore the importance of tailoring treatment plans to individual patient profiles, balancing therapeutic benefits against potential risks to optimize clinical outcomes.

Discussion

This study provides a comprehensive evidence-based comparison of the safety and efficacy outcomes of oncolytic virotherapy in patients with various malignancies. Our analysis of safety endpoints suggests that oncolytic virotherapy is associated with an increased risk of adverse events, encompassing effects across multiple organs and systems. Specifically, the risk of all-grade and severe adverse events related to general disorders and administration site conditions was significantly elevated in the virotherapy-treated group. While oncolytic viruses are generally considered well-tolerated, the potential for severe adverse events should not be underestimated.[47,48] Notably, the infusion of oncolytic viruses can elicit a systemic immune response, potentially resulting in off-target effects and normal tissue damage due to immune system dysregulation. Through head-to-head pairwise analyses, we identified that the risk of all-grade adverse events was linked to virotherapy-based treatment strategies, while severe adverse events appeared to be influenced by virus-derived classifications, possibly reflecting variations in immune activation mechanisms. Furthermore, factors such as patient age, tumor type, and routes of viral administration significantly contributed to the occurrence of adverse events. Given that most of cancers occur in patients aged 40 years and older, and recognizing the immunological differences between age groups as highlighted in oncology research, age selectivity remains a critical consideration.[49–51] Additionally, innate immune responses to oncolytic virus infections exhibit variability across different virus classifications, emphasizing the heterogeneity in treatment outcomes.[52–54] These findings underscore the imperative to investigate the relationships between agent-induced phenotypes and underlying molecular mechanisms and to identify predictive biomarkers for toxicity. Such efforts could mitigate the risks of potentially hazardous treatment combinations, optimize the therapeutic potential of oncolytic virotherapy, and enhance patients' long-term quality of life. Consistent with this, the early identification of risk factors and the implementation of appropriate management strategies are essential for ensuring patient safety and maximizing clinical benefits.

As clinical efficacy endpoints, the objective response rate and disease control rate were employed to evaluate both the direct tumor-lytic and tumor-static effects of oncolytic virotherapy. Our analyses revealed that the efficacy of virotherapy is influenced by treatment strategies and virus-derived classifications. Current advancements in understanding tumor classification,

particularly in relation to tumor-immune system interactions, have significantly expanded. Regardless of whether tumors are immune-infiltrated or immune-silent, short-term antitumor responses can be attributed to the intrinsic oncolytic activities of viruses and the antitumor immune responses they elicit. This evidence supports the potential of oncolytic viruses to mitigate the immunosuppressive tumor microenvironment and convert immune-silent tumors into immune-infiltrated phenotypes. Several factors may explain the observed differences in short-term therapeutic effects. First, exploratory analyses suggest that the structural and genomic characteristics of oncolytic viruses play a pivotal role in their efficacy.[55–57] Second, tumor immune profiles significantly influence therapeutic outcomes, with immune-infiltrated tumors demonstrating enhanced responses due to the synergistic effects of virotherapy. Conversely, immune-silent tumors, characterized by low or absent tumor-infiltrating lymphocytes, appear less responsive.[58–64] This raises the possibility that persistent, detrimental factors may counteract the benefits of oncolytic virotherapy, necessitating further investigation into the underlying mechanisms. Third, while delivery methods did not emerge as a primary cause of efficacy discrepancies, our findings indirectly suggest that the oncolytic viruses used in the included trials maintained their effectiveness during intravenous administration. This is likely attributable to specific engineering that circumvents attenuation by pre-existing blood factors or abnormal tumor vasculature structures.[58,65,66] Fourth, previous studies have reported that sex can modulate cancer prognosis in immunotherapy settings, highlighting potential sex-immune system interrelationships.[67,68] In our subgroup and meta-regression analyses, sex differences were identified as potential confounders. As an emerging immunotherapy, the sex-related efficacy of oncolytic viruses warrants objective evaluation in future trials and mechanistic studies. Moreover, the line of treatment remains a critical factor. Most patients in the included studies had undergone prior systemic therapies, such as chemotherapy or radiotherapy, which are non-targeted and may impair innate immune cells, thereby reducing antitumor immunity.[69,70] Developing alternative therapeutic strategies for these patients is imperative. Additionally, recent studies suggest that the broad-spectrum infectivity of oncolytic viruses may inadvertently stimulate both innate and adaptive antiviral immunity, potentially diminishing their antitumor effects.[58,71] This dual immune activation could contribute to poorer prognostic outcomes, yet the relationship between antiviral responses and clinical prognosis remains poorly understood. Addressing these gaps in knowledge through future research is essential to elucidate the mechanisms balancing antiviral and antitumor immune responses.

Treatments strategies were categorized based on clinical applicability and mechanistic synergy. Oncolytic virus monotherapy (e.g., T-VEC) serves as a critical benchmark for evaluating the intrinsic efficacy and safety of the oncolytic viruses without the confounding effects of additional interventions.[72] The rationale for combining oncolytic viruses with immunotherapy lies in leveraging their synergistic potential. Oncolytic viruses can modulate the tumor microenvironment, rendering it more susceptible to immunotherapeutic agents. Regimens involving two or more immunotherapies were classified separately due to their distinct toxicity profiles.[73] Furthermore, combinations with chemotherapy or radiotherapy were categorized to assess the potential for enhanced tumor cell eradication and improved clinical outcomes when oncolytic viruses are paired with traditional therapeutic modalities.[74]

The virus families analyzed in this study were grouped based on biological and clinical homogeneity, encompassing *Herpesviridae*, *Adenoviridae*, *Picornaviridae*, *Reoviridae*, and *Poxviridae*. *Herpesviridae* viruses exhibit robust replicative capacity and potent oncolytic activity, enabling efficient tumor cell lysis. Their large genomes facilitate extensive genetic modifications to express therapeutic transgenes, enhancing antitumor efficacy. *Adenoviridae* viruses, with their superior transduction efficiency and broad cellular tropism, achieve robust infection across diverse tumor types and are amenable to engineering multiple therapeutic transgenes to augment oncolytic potential. Among RNA viruses, *Picornaviridae* members are characterized by rapid replication kinetics and intrinsic tumor selectivity, which can be further optimized through genetic targeting to enhance tumor-specific cytotoxicity. *Reoviridae* viruses naturally target transformed cells, particularly those with activated RAS pathways, enabling selective cancer cell destruction without genetic modification. *Poxviridae* viruses provide exceptional genomic payload capacity, allowing simultaneous delivery of multiple immunomodulatory transgenes, making them ideal for combination therapies with immune checkpoint inhibitors. The stratification into monotherapy and combination therapy regimens allows for a systematic evaluation of the synergistic effects of oncolytic viruses when combined with complementary treatment modalities. This approach facilitates the quantification of incremental benefits derived from multimodal therapeutic strategies, advancing our understanding of their clinical utility.[75–77]

Oncolytic virotherapy has demonstrated significant potential in cancer treatment; however, its clinical application faces several challenges, including issues related to viral vector production, treatment costs, and real-world feasibility. First, the production of oncolytic viruses must adhere to Good Manufacturing Practice (GMP) standards, yet only a limited number of institutions worldwide possess the capability for large-scale production.[72,78,79] Moreover, the development and transportation costs of genetically engineered viruses

can reach hundreds of millions of dollars, leading to clinical treatment costs that are considerably higher than those of traditional therapies.[56,80] Although the per-injection cost of marketed oncolytic viruses is relatively low, the necessity for multiple injections results in annual treatment expenses of approximately $4200.[79] In comparison, chimeric antigen receptor (CAR) T-cell therapy entails significantly higher costs, with a single dose priced at $140,000, highlighting a notable cost advantage of oncolytic virotherapy.[81,82] Second, most clinical trials involving oncolytic viruses excluded immunocompromised patients, such as those with HIV or autoimmune diseases. This exclusion limits the availability of real-world clinical data for these populations, restricting the scope of current applications. Third, combination treatment strategies incorporating oncolytic viruses require optimization, particularly regarding sequencing and dosage standardization. Without established protocols, some combinations may increase the risk of adverse reactions, posing further challenges to clinical implementation.

In assessing the risk of bias, we employed multiple tools tailored to different study designs, including the Cochrane ROB2, ROBINS-I, and MINORS tools. This comprehensive approach facilitated the identification and quantification of potential biases across studies.[25,26] For randomized controlled trials, the ROB2 tool identified issues such as inadequate allocation concealment and lack of blinding, which may lead to overestimated effect sizes. For non-randomized trials, the ROBINS-I tool highlighted selection and information biases that could affect relative efficacy evaluations of treatment strategies. In single-arm trials and cohort studies, we utilized the MINORS tool to assess bias risk.[27] The MINORS tool systematically evaluates study quality, categorizing studies as high, moderate, or low quality based on their scores. Among the included studies, 154 were single-arm trials, of which 142 achieved scores between 12 and 14, indicating rigorous methodology and low risk of bias. The remaining 12 studies scored between 8 and 11, representing moderate-quality studies with some methodological limitations but retaining reference value. Common issues identified by the MINORS tool included inadequate follow-up and incomplete data reporting, which may contribute to heterogeneity.[83,84] Overall, while the diverse range of bias assessment tools provided a robust framework for evaluating study quality, the findings underscore the importance of addressing methodological limitations to enhance the reliability and interpretability of meta-analyses in oncolytic virotherapy research.

The primary strengths of this study lie in its comprehensive and quantitative approach. The data were derived exclusively from published clinical trials, ensuring reliability and a robust basis for analysis. The outcomes were evaluated using well-defined endpoints, allowing for a precise assessment of adverse events. A Bayesian network meta-analysis was conducted to address research questions that could not be adequately resolved through conventional pairwise meta-analyses. While traditional pairwise analyses compared virotherapy with a control, the network meta-analysis enabled the simultaneous comparison of multiple treatment strategies through indirect evidence synthesis. This approach provided clinically relevant rankings using SUCRA values, which serve as valuable tools for clinicians when selecting optimal treatment strategies among multiple options. The Bayesian framework facilitated a thorough evaluation of transitivity via node-splitting analysis and consistency models, enhancing the validity of cross-trial comparisons, an advantage not achievable with standard meta-analyses. For outcomes where between-study variance was observed in the network meta-analyses, Bayesian meta-regression was employed to explore individual empirical differences and their association with relevant covariates. Sensitivity analyses further confirmed the robustness of the results. These findings provide a solid foundation for evidence-based clinical decision-making and the development of personalized treatment regimens tailored to various clinical scenarios. Additionally, the insights derived from this analysis contribute to advancing scientific research and promoting ongoing basic investigations into oncolytic virotherapy. With numerous ongoing studies in this field, the integration of data from preclinical research and clinical trials will expand the understanding of oncolytic viruses and support their optimized application in clinical practice.

The primary limitation of this analysis is the potential for investigator bias in the original trials, which restricts the generalizability of the findings to patients meeting the specific eligibility criteria of those studies. Additionally, small-study effects were evident, with studies involving smaller sample sizes exhibiting wider credible intervals for both severe adverse events and efficacy outcomes. To mitigate these effects, the network meta-analysis employed a Bayesian framework with conservative priors, and sensitivity analyses were conducted to confirm the robustness of the conclusions. Another limitation arises from the exclusion of non-English publications, which may introduce potential language bias. To address this concern, we systematically evaluated the geographic distribution of the included trials and found that a substantial number originated from non-English-speaking regions such as China, Japan, and Germany. Importantly, non-English publications represented only a minimal proportion of eligible studies. Screening revealed that 98% of qualifying studies were published in English, indicating that the exclusion of non-English literature likely has a negligible impact on the reliability of the conclusions.[85,86] A notable challenge in interpreting the results stems from the variability in prior treatments received by patients before trial enrollment, which differed

across studies. Although rigorous inclusion criteria were implemented to standardize baseline characteristics, the treatment line remains an important consideration when evaluating the findings. Consequently, the prognostic effects of oncolytic virotherapy should be interpreted with caution. Furthermore, this meta-analysis relied on published aggregate data rather than individual patient data (IPD), which limits the ability to fully account for the influence of other potential variables on the outcomes. To address these limitations, future research could implement the following measures: Increasing study sample sizes to reduce small-study effects and enhance the stability of results. Standardizing treatment lines for cancer patients to minimize their impact on study outcomes. Utilizing IPD analyses to comprehensively assess the influence of patient-level variables on efficacy and safety. Conducting multicenter studies to improve sample diversity, thereby increasing the generalizability of findings. Lastly, most existing studies have relatively short follow-up periods.[87,88] Long-term studies are required to evaluate the enduring safety and efficacy of oncolytic virotherapy. Although combination therapy strategies have demonstrated superior objective response rates and disease control rates, further research is warranted to optimize these regimens for clinical application.

This meta-analysis highlights the efficacy and safety of oncolytic virotherapy in cancer treatment, underscoring its potential as both monotherapy and in combination regimens. Frequently reported adverse events included fatigue, pyrexia, fever, and chills, with severe adverse events such as neutropenia and lymphocytopenia primarily observed in combination therapies. While the addition of chemotherapy or radiotherapy to oncolytic virotherapy improved disease control rates, it also significantly increased toxicity risks. In contrast, virotherapy combined with immunotherapy demonstrated a favorable safety profile and clinical benefits, particularly with regimens incorporating multiple immunotherapies or *reovirus*/*poxvirus*-based approaches. The therapeutic efficacy of oncolytic virotherapy varied across virus types, delivery routes, and patient demographics. Combination strategies, especially those based on *herpes simplex virus*, achieved higher objective response rates and disease control rates, with intratumoral injection showing superior efficacy. Adults responded better to virotherapy than children, and monotherapy proved to be an effective alternative for patients unresponsive to immunotherapy. These findings emphasize the importance of personalized treatment strategies, balancing efficacy with potential risks, to optimize clinical outcomes.

Contributors

XC, JZ, and HL conceived the study project. PL and LL designed the study, collected literatures and accessed the quality of trials. LL, JZ, and HL performed the data extraction and checking. PL and LL did the statistical analysis and created the figures and tables. PL and LL verified the underlying data. All authors contributed to interpretation of results. PL and HL wrote the first draft of the report. All authors read and approved the final version of the manuscript.

Data sharing statement

All data analyzed during this study are included in the published article and its supplementary information files.

Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2025.103362.

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