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Designer nanocarriers for navigating the systemic delivery of oncolytic viruses

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- **Abstract:**
  Nanotechnology is paving the way for new carrier systems designed to overcome the greatest challenges of oncolytic virotherapy (OV); systemic administration and subsequent implications of immune responses and specific cell binding and entry. Systemic administration of oncolytic agents is vital for disseminated neoplasms, however transition of nanoparticles (NP) to virotherapy has yielded modest results. Their success relies on how they navigate the merry-go-round of often-contradictory phases of nanoparticle delivery: circulatory longevity, tissue permeation and cellular interaction, with many studies postulating design features optimal for each phase. This review discusses the optimal design of nanoparticles for OV transport within these phases, to determine whether improved virotherapeutic efficacy lies in the pharmacokinetic/pharmacodynamics characteristics of the NP-OV complexes rather than manipulation of the virus and targeting ligands.

- **Lay abstract:** optional – N/A
- **Graphical abstract:** optional – N/A
- **Video abstract:** optional – N/A

- **Keywords:** Oncolytic virotherapy, nanomedicine, nanoparticles, nanotargeting, immunotherapy, magnetic guidance

- **Main body of text:**

1. Introduction

Cancer killing oncolytic viruses (OV) are a promising treatment modality for cancer. However, variable clinical response rates [1] have shifted the impetus towards various carrier strategies for their systemic delivery including liposomes, polymers, cell carriers and metallic nanoparticles to name a few. Carrier systems are not new in the field of oncology with many small molecule inhibitors (SMIs) embracing their advantages including biocompatibility, increased endocytosis, solubility and extended circulation time compared to free anticancer drugs, whilst simultaneously reducing systemic toxicity [2]. Successful examples include liposomal doxorubicin and nanoparticle albumin bound paclitaxel, which have progressed to the clinic. Viruses face similar pharmacokinetic and pharmacodynamic (PK/PD) challenges to SMI's which have been mitigated to a certain extent by the transfer of carrier systems to the field of virotherapy. To successfully reach target tumour cells by a passive system the ideal therapeutic agent must traverse three major phases for nanoparticle drug delivery for oncology; systemic circulation and reticuloendothelial system (RES) interaction, extravasation and tumour penetration and interaction with

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target cells. The physical characteristics (shape and size) and chemical characteristics (composition and charge) of nanoparticles are significant determinants of their functionalities within these three phases yet they have contradictory requirements leading to a paradoxical merry-go-round (Figure 1) that often requires sacrifice of design features optimal for at least one of the other phases. From this perspective it is even more striking how similar in both composition and mode of action the current raft of available nanoparticles are (Figure 2). We will explore whether this represents a conscious decision by investigators to focus on one phase they believe to be the most important or whether other limiting factors such as fabrication technologies have driven these decisions. This review will discuss the PK/PD of nanoparticles and whether this knowledge can be applied for the generation of virotherapeutic complexes endeavouring to reconcile the opposing determinants of their success.

2. Pharmacokinetics of nanoparticles for oncology

The influence that the characteristics of nanoparticles have on their transport and interaction with cells is well known and has been extensively reviewed by Ernsting et al [3] within the context of oncology. The key points are represented in Figure 3 and briefly discussed below.

Morphology

Size and shape influences nanoparticle transport behaviour including how they interact with the endothelial cells of blood vessels [4] and capillarys wall for extravasation before being removed from the blood either by the RES or filtered by the lungs, liver and spleen. Particles larger than 5µm are trapped within the capillary beds of the liver and between 1-5µm they are phagocytosed by Kupffer cells [5]. Circulating nanoparticles exceeding 100nm in diameter are rapidly phagocytosed by hepatic and splenic macrophages, as opsonisation by serum proteins increases with size [3, 6]. However, to enhance extravasation into tissues, nanoparticles approaching 100nm are more likely to marginate towards the periphery of the blood vessel [7]. Radiolabelled metal organic frameworks (MOFs) of 60nm demonstrated longer blood circulation and over 50% higher tumour accumulation than 130 nm MOFs [8]. Formation of nanoparticle aggregates is also a concern for nanodrugs due to the risk of embolism and changes in biodistribution, with aggregates of polystyrene aggregates demonstrating a higher propensity for the reticuloendothelial systems [3, 9]. However, aggregation of aerosolised gold nanoparticles demonstrated

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significantly faster cellular uptake than single AuNPs at the air-blood barrier interface using a multicellular lung system [10] once again forcing investigators onto the merry-go-round of decision-making as to which delivery phase is most important to them (Figure 1).

Once within the tissue, size also affects permeation and cell internalisation. Small nanoparticles show high permeation rate but also are rapidly cleared from the tumour by RES leading to poor accumulation (Figure 3) [11]. Never is size more important than when considering transport across the blood-brain barrier for treatment of brain neoplasms. A recent study in a mouse model of orthotopic glioblastoma multiforme (GBM) demonstrated higher distribution of gold nanoparticles (AuNPs) within tumorous tissue compared to normal tissue when perfused via the carotid artery. Pertinently, nanoparticle size affected the permeation of nanoparticles with 10nm AuNPs widely distributed throughout the brain tumour, whereas 50 and 100 nm AuNPs were located near the blood vessels [12]. By using carotid infusion, the investigators were able to bypass renal filtration, however, clinically this would be considered a highly invasive procedure with usual standard of care recommending intravenous administration therefore exposing these particles to rapid elimination from the circulation.

NP size may affect the uptake efficiency and kinetics, the internalisation mechanism (eg. clathrin or caveolin mediated endocytosis and phagocytosis) [13] and also the subcellular distribution. A size-dependent uptake in different cell lines has been observed as well as size dependent cytotoxicity which is extensively reviewed by Shang et al [14]. They concluded that the optimal size for active cellular uptake required a NP core size of 30-50nm. However this is within the range that NP's are rapidly cleared by the RES following systemic administration. Recently it has also been postulated that tumour volume can selectively change tumour uptake of nanoparticles of varying size and that this depends on the frequency of interaction of particles with the perivascular extracellular matrix for smaller nanoparticles, whereas transport of larger nanomaterials is dominated by Brownian motion [15], adding a further layer of complexity to nanocarrier design.

There is also evidence that the size of NPs can influence the therapeutic effect. Naked AuNPs can inhibit the function of pro-angiogenic heparin-binding growth factors (HB-GFs) and subsequent intracellular signalling events. Using AuNPs of 10, 20 and 30nm, investigators demonstrated that the specific inhibitory effects of AuNPs towards HB-GFs are size dependent, bigger nanoparticles being more efficacious at inhibiting proliferation of HUVEC and NIH3T3 cells *in vitro* (100% inhibition at 5nM/L) [16].

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The shape effect is also known to influence fluid dynamics including lateral drift of particles towards the blood vessel wall, how particles interact with tumour capillaries during transport and their role in intracellular uptake [17]. However, it is only in recent years that this characteristic has begun to draw interest, most likely previously hindered by the lack of fabrication technology. In terms of circulation time, investigations have demonstrated, using Tobacco Mosaic Virus (TMV) in mice, that spherical NPs are cleared more rapidly from tissues in comparison to nano-rods of the same chemical composition [18]. Moreover, TMV has been used to generate a computational simulation to model nanoparticle diffusion within a small segment of solid tumour between capillaries [19]. They concluded that smaller aspect ratios (AR) (rod-shaped) NPs have higher diffusion and accumulate more easily in the tumour tissue, however higher aspect ratio NPs possess enhanced margination (the ability of particles to migrate towards blood vessel walls in blood flow), increased transport across tissue membranes and reduced clearance by phagocytosis. Likewise, Lee et al (2009) observed that whilst elongated particles (small AR) had a greater propensity to marginate in linear laminar flow, this could only be achieved through the application of external forces (gravitational, magnetic) within blood microcirculation [20]. Internalisation of particles was dependent on both the shape and absolute size and/or volume of the particle with larger rod-shaped particles internalised by HeLa cells 4 times faster than symmetrical particles and smaller rod-shaped particles [21], possibly due to the larger surface areas in contact with the cell membrane. Spherical metallic nanoparticles displayed greater cytotoxicity compared to rod-shaped [22] and star-shaped [23] nanoparticles in human skin fibroblasts although results from these studies could also be attributed to size and surface chemistry.

Surface characteristics

To overcome the general lack of specificity of nanoparticles and poor biodistribution a myriad of coatings exist purporting enhanced functionalisation capabilities, biocompatibility and aggregation reduction. The most successful strategy in this endeavor has been the wide-spread use of polyethylene glycol (PEG), a water-soluble synthetic polymer that creates a stable hydration layer resistant to protein adsorptions to underlying surfaces. However an increase in PEGylated proteins and PEG-modified NPs including liposomes and micelles has seen a correlation of anti-PEG antibodies with loss of therapeutic efficacy both in the clinic [24, 25] and in animal models [26, 27]. What once was used as a shield against opsonisation and immunosurveillance is now a recognised determinant for their clearance [28] resulting in loss of

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therapeutic efficacy and increase in adverse effects. Biomimetic coatings may represent the future, where nanoparticles are recognised as "self" using membrane materials such as red blood cell membrane-coated nanoparticles which may enhance glioma therapy efficacy [29] and has demonstrated 29% overall retention after 24 hours within the circulation of mice compared to bare NPs that showed negligible signal at the first 2 minute timepoint [30]. Surface coatings can also change the charge of the NPs and exert a major effect on their properties including drug loading and release rates, systemic circulation and absorption [31]. As shown in Figure 3, charged NPs are more prone to serum protein opsonisation and interactions with negatively charged plasma membranes compared to neutral NPs yet cationic NPs display enhanced cellular uptake, once again forcing the investigator to choose between enhanced circulatory time or target cell interactions (Figure 1).

3. Virotherapy

Dysfunctional cellular processes that are the markers of neoplasms provide the ideal environment for viral infection including sustained proliferation, resistance to cell death, the evasion of growth suppressors and immune destruction, genome instability and DNA damage stress [32]. The attraction to virotherapy in comparison to conventional treatments is the unique ability of OV to only kill cancer cells. For example, inherently selective RNA viruses such as the measles virus vaccine strain MV_SPUD, reovirus and Newcastle disease virus take advantage of tumour cell resistance to interferon [33], whilst vaccinia virus, adenovirus, Herpes simplex virus (HSV) and polioviruses exploit the deletion of viral genes [34-37], that are necessary for replication in normal cells but expendable in cancer cells. Further advantages, summarized in Table 1, include the OV ability to target multiple oncogenic pathways and use multiple means for cytotoxicity thereby avoiding cross-resistance encountered in standard anticancer therapies with minimal side effects due to tumour specific replication. Whilst they are not without their risks (including virus replication, insertional mutagenesis, loss of specificity, immunogenicity and adverse effects) [38, 39] a predicted compound annual growth rate of 24.9% between 2018-2025 [40] and multitude of companies and academic sectors either emerging or investing in this field [1] is testament to the exciting success of T-Vec as a treatment for melanoma [41-43] and more recently the clinical trial involving safety assessments of Parvovirus as a treatment for glioblastoma [44]. A number of studies have provided evidence for the natural oncolytic activity of viruses and have been extensively reviewed for

intratumoral administration elsewhere [45-48], but crucially, it is well understood that to be clinically relevant systemic delivery is optimum. Not only is it a relatively simple procedure but could also potentially facilitate treatment of disseminated disease and hard to reach tumours including those beyond the blood brain barrier. Whilst systemic administration of viruses has achieved efficacious results preclinically, including vesicular stomatitis virus [49-52], Newcastle disease virus [53, 54], reovirus [55], lentivirus [56] and herpes simplex virus [57, 58], they have translated poorly into humans [59, 60].

Despite the many advantages coupled with a spectrum of virus types, tropism and targeting pathways there are a number of obstacles to overcome to ensure clinical translation against both solid and disseminated tumours**.** These include the role of innate and adaptive immunity; the effect of viral tropism towards the liver resulting in liver toxicity and clearance and the physical barriers including tumour extracellular matrix and limited extravasation of OV's due to high interstitial fluid pressure within the tumour. The heterogeneity in the clinical response to OV therapies also suggests that a one-pronged approach to treatment may facilitate relapse and metastasis in heterogenous tumours, through the incomplete eradication of neoplastic cells [61]. Combination therapies have demonstrated synergistic antitumour effects [62] for example the facilitation of virion assembly and upregulation of CAR expression by paclitaxel (PTX) for Ad internalisation elicited by PTX-conjugated micelle-coated oncolytic adenovirus [63] whilst Tong et al., demonstrated that both intratumoral heterogeneity as well as extensive interpatient heterogeneity impacts the potential of Maraba virus as an oncolytic agent for ovarian cancer [64]. Oncolytic virotherapy therefore exists in a paradoxical situation whereby many of their attractive features also serve in their destruction e.g. immune responses designed to remove unrecognisable microorganisms yet also augment immune cell death (ICD) and cancer cell destruction.

Whilst genetic engineering of viruses has sought to overcome the problems associated with cell targeting and entry, this technique requires extensive modification of the capsid to incorporate new moieties, which is a laborious process and risks generating non-infectious or dysfunctional virus. In comparison, chemical engineering is simple and straightforward, since nanomaterials can be complexed with viruses through chemical conjugation or electrostatic interactions. It is this versatility that has attracted many investigators to utilise the advantages that NPs such as liposomes, polymers and cell carriers convey, yet despite the wealth of data described earlier regarding PK/PD of these NPs, there is a distinct pattern of strategies that have emerged.

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4. Strategies employed by nanoparticles to enhance virotherapy delivery

As mentioned, there are three predominant stages for systemic NP delivery in oncology which has led to the three most important modifiable strategies for increasing concentrations of therapeutics at tumour targets; 1) shielding within the circulation; 2) tumour targeting; and 3) cell entry (Figure 4).

Shielding

Evading the humoral immune response is one of the biggest challenges facing systemic administration of OV's such that failure to do so will result in virus neutralisation before they have reached the target. The liver and spleen rapidly clear systemically administered viruses from the circulation by opsonisation with antibodies, complement and coagulation factors resulting in sequestration by the mononuclear phagocytic system (MPS). Yet viral replication, the release of pro-inflammatory cytokines and the accumulation of inflammatory cells to the tumour microenvironment (TME) are critical to tumour cell death [65, 66]. Consequently, an interplay between OV activity and an inflammatory response is believed to aid in the therapeutic efficacy of OVs in an *in vivo* environment. Some viruses have managed to overcome these challenges by disguising themselves within host cells for delivery to their target cell population including the human immune deficient virus (HIV) which utilises dendritic cells and macrophages that naturally migrate to the lymph nodes for delivery to CD4+ T cells [67]. Cell to cell spread of viruses also enables evasion of antibody neutralisation [68, 69]. Investigators are again taking inspiration from these evolved survival mechanisms to optimise cell carrier systems in a number of tumour models as a way of disguising OV from host defences prior to deposition at its target cell population (Figure 3). Avoiding virus sequestration by the MPS is achievable by shielding virus particles with chemically modified coat proteins. Probably one of the most studied uses of nanoparticles for virotherapy is the decoration of viruses with various polymers/micelles [63, 70-74], dendrimers [75, 76], liposomes [77-80], and cells [81, 82]. Alternatively, pre-administration of compounds designed to deplete serum factors (Cobra venom factor [83], cyclophosphamide [84, 85]), saturate scavenger receptors (polyinosinic acid) [86, 87] and diminish splenic macrophages using clodronate-loaded liposomes [88, 89] have been used to enhance therapeutic outcome by downregulating specific compartments of the immune system. Ultimately these strategies require extensive optimisation and understanding of the concomitant effects.

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Shielding of the viruses via masking their surfaces with NPs requires some method of complexation of the two components. The zeta potential/surface charge is therefore probably one of the most important factors for carrier systems that do not involve encapsulation of viruses. To the best of our knowledge, complexation of viruses with NPs has been predominantly via spontaneous electrostatic interactions for the formation of safe complexes at a charge neutralised ratio [90-92]. However, in these examples, very little is known regarding their final shape, uniformity and stability with binding success often only assessed by changes in the net surface charge and particle size. There are very few examples where the coexistence of the various components have been verified by investigating chemical characteristics on surface composition and the bond types participating in the complexation [93-95]. One also needs to consider how strong these interactions are and whether they are displaced following injection into a turbulent fluidics system and displacement by the presence of other proteins such as serum albumin.

It is interesting that electrostatic interaction is the only method that has been utilised by investigators with examples for exploiting these interactions further by the addition of cationic coatings (eg TAT peptide), especially when covalent binding may provide a more stable bond and offers flexibility in terms of available residues and reversible linkers that can be activated under certain conditions and thereby act as triggers for more targeted delivery. One such reason may be evidence that bioconjugation in nanoparticle-based drug delivery systems alters their properties with glutaraldehyde protein cross-linking influencing the induced antibody responses at several levels *in vivo* [96] which would be an important consideration due to the inter-dependence of the immune response in the efficacy of virotherapy.

Tumour targeting/recognition

If OV's manage to avoid detection in the systemic circulation their next challenge is to accumulate at their tumour target (Figure 4). This passive transport and non-specific accumulation of drugs in solid tumours has long been attributed to the enhanced permeability and retention (EPR) effect whereby a combination of their so called "leaky" vasculature for tissue entry together with a lack of well-defined lymphatic systems results in increased retention times [97]. Strategies for a more specific method of targeting tumour cells (often called active targeting), is to either recognise a determinant expressed directly on/by the tumour cell, by recognising the microenvironment created by the tumour (homing macrophages to

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hypoxic areas [82], trafficking mesenchymal stem cells to areas of tumour-associated cytokine production [98, 99] or endothelial cells to tumour-associated angiogenesis [100]), or by exploiting intrinsic trafficking to specific tissues or organs (e.g. nonspecific T cells which naturally home to lymphoid organs) (Figure 3). One mechanistic explanation for intrinsic tumour tropism of viruses is linked to the inability of many cancer cells to respond properly to pro-inflammatory or antiviral cytokines [101]. For example, defects in the interferon (IFN) responses of cancer cells allows VSV to replicate, even in the presence of IFN where in normal primary mouse cells, the replication of VSV is strongly inhibited by IFN. One common method of genetically modifying an oncolytic virus candidate to increase tumour tropism is to delete or modify the viral genes responsible for countering cytokine-mediated immune responses such as members of the interleukin (IL) family, erythropoietin and interferons to name a few [102].

Functionalising the surfaces of NPs rather than modifying the viruses is also a common approach for re-targeting these drug delivery systems including the addition of ligands such as luteinizing hormone releasing hormone receptors [103] in breast cancer and anti-CD47 in pancreatic cancer cell targeting [104]. Redirection of virion-liposome complexes containing Moloney leukaemia virus (MMLV) to vascular endothelium by incorporation of antibodies for the endocytic receptors CD71 and CD62E/P [105] has been achieved and ovarian cancer growth has been cessated by systemic administration of a liposome-encapsulated adenovirus-encoding endostatin which decreased angiogenesis and increased tumour apoptosis [79]. Similarly, oncolytic adenovirus complexed with EGFR targeted dendrimer can be efficiently internalised by EGFR positive tumours [75], further validating the benefits of this strategy. Ultimately even these ligand-bearing NP-OV complexes rely on passive transport and whilst the EPR effect is widely held to increase nanotherapeutic delivery to tumours over normal organs owing to their defective tumour vasculature, the heterogeneity of the EPR effect in cancer [106] often offers less than a 2-fold increase in nanodrug delivery compared with critical normal organs, resulting in drug concentrations that are not sufficient for curing most cancers [107].

Nano entry into cell targets

The final hurdle for OV's once they have reached their tumour target is the mechanisms for cell entry (Figure 4). Whilst the natural tropism towards tumour cells by certain viruses is selected by their

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phenotype (immortality, DNA instability etc), cell entry relies on specific signalling pathways i.e. CAR expression is required for internalisation of adenoviruses [108] whilst glycoprotein D is an essential component of HSV-1 entry apparatus [109]. These signalling pathways can be enhanced for either increased recognition or enhanced viral replication. For example a genetically modified version of the measles virus (MV) vaccine strain which already demonstrated tropism towards many different types of cancer cells through the highly expressed CD46 receptors induces greater oncolytic activity against renal cancer cells [71]. Conversely, liposomal mediated entry of viruses are independent of viral receptors as they attach and reform with the cell membrane. This entry method has proven to enhance infectivity of liposomal HSV-1 even in the presence of neutralising antibodies following systemic administration for liver metastases [78]. Magnetofection is a membrane-receptor independent mechanism for hard-to-transfect biological models that uses a magnetic field to transfect cells [110], this technique has also demonstrated enhanced adenovirus uptake and increased time to full oncolytic effect *in vitro* and *in vivo* with consequential significant inhibition of tumour growth in a murine xenograft of human pancreatic carcinoma following intratumoural administration [90].

Whilst cell carriers may provide OV's with the ideal disguise for avoiding immunosurveillance, their successful delivery is complicated by a number of considerations including the kinetics of viral replication and release and kinetics of trafficking of the cell carrier from the site of injection to the tumour location. Cell carriers that support viral replication provide the added advantage of amplification overcoming issues including limits of clinical grade virus production and increase the therapeutic index and the likeliness of overcoming neutralising antibodies [82, 111]. Timing of replication and release is important as accumulation of viral proteins at the surface of the infected cell (e.g. VSV G protein) or creation of IHC complexes can alert the immune system resulting in premature clearance. Mitigation of this risk could involve either co-ordination of the lag time following initial viral infection of a cell and release of progeny virions with transport to target tissue or initiation of viral replication and release only at the targeted tumour. Shielding viruses within immune cells such as T cells avoids eliciting an immune response but they do not support all viruses and can be refractory to viral infection *in vivo* [112]. Mesenchymal stem cells can also support therapeutic adenoviral replication [113] yet more recently neural stem cells have demonstrated enhanced viral loading with significant increases in survival rates administered intracranially in an orthotopic glioma model [114] as well as significant reduction in omental tumour burden in an orthotopic model of ovarian cancer [115]. Whilst promising, both these examples utilised

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direct administration of the treatment to the tumour site, not to mention the extraction of neural cells requiring invasive stereotactical surgery.

The toxic environments associated with tumours also influence cell entry and therapeutic effects for example hypoxia, which is associated with resistance to radiotherapy and chemotherapy despite enhanced delivery of these drugs via carriers. Liposomal formulations of SMI's doxorubicin [116], daunorubicin [117] and paclitaxel [118] have demonstrated success in the clinic although augmentation of efficacy above drug alone is debatable [119, 120]. This could be attributed to the characteristics of the carrier including their size (too large and they are captured by the reticuloendothelial system or too small and they are excreted in urine). In addition, reduced cellular uptake and cellular adaptations can also compromise the effectiveness of the chemotherapy [121] due to exposure to toxic conditions. Investigators have sought to adapt these situations to their advantage by using them as molecular switches for the release or activation of therapies e.g. Hypoxia activated pro-drugs (HAPs) such as TH-302 and PR-104, reviewed by Baran and Konopleva [122]. Fortunately, hypoxia seems to exert little or no effect on the replication of some oncolytic viruses [123]. Replication of adenoviruses from both groups B and C is inhibited, yet replication of the herpes simplex virus G207 strain was enhanced in brain and breast cancer cell lines by a 74% increase in cytotoxicity of hypoxic MCF 7 cells [124] and a 3.6-fold increase in G207 viral titres by hypoxic U87 glioblastoma cells [125]. This idea of activating switches has also been applied to MNP's for controlled drug release. Thermodegradation of polymer caps by application of an oscillating magnetic field to mesoporous silica nanoparticles has unblocked nanochannels containing drugs [126, 127], thereby releasing therapeutic cargo on demand in the desired location. Applying this strategy to OV could provide protection against immunosurveillance and allow targeted release thereby potentially increasing biocompatibility and efficacy.

Despite these strategies for enhanced virus protection and target recognition, perhaps the biggest limitation for any systemically administered therapy is its reliance on passive delivery with evidence demonstrating that nanoparticles (NPs) displaying decreased blood circulation time usually display concomitant reduced tumour uptake and efficacy [3]. It is therefore feasible that the similarities displayed by the current raft of available NPs (spherical, 80-120nm diameter, membranous) is a reflection of this (Figure 2). Thus would an active guidance/steering system allow for more radical designs of NP-OV

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complexes by incorporating a more holistic approach encompassing the optimum features from all three strategies (Figure 4).

Designer OV-nanocomplexes

Utilisation of viruses as a treatment modality in cancer have so far focused on viral tropism to specific tumour types for intratumoural administration or the addition of specific receptors for target recognition following systemic administration. Despite the multitude of drug delivery systems available they are all based on this principle of additional recognition moieties to overcome the limitations of their passive transport and discriminate between healthy and neoplastic cells**.** The literature therefore suggests that there is a requirement that the virus be "matched" to its target in order to achieve efficacy. However, these solutions lack a propelling force to a specific area as well as to penetrate tumours beyond diffusion limits. Systemic administration of naked virus as well as functionalised nanoparticles are inefficient as they still rely on the EPR bias (whereby the tumour vasculature is considered more "leaky" than that of normal organs) to navigate the vasculature. Perhaps it is therefore unsurprising that despite the wealth of knowledge regarding the pharmacokinetics of NPs within each phase of NP drug delivery there is a trend towards spherical particles of 80-130 nm diameter with lipid-based outer coatings (Figure 2). Would application of an external driving force allow radical divergence away from these parameters and translate to improved efficacy?

Application of external magnets have sought to overcome this problem of passive targeting. Magnetic chemotherapy has improved drug delivery for monotherapies such as doxorubicin [128], photothermal ablation with cetuximab [129] and melanin [130], dual targeting combining ligands with therapeutics [131], as well as utilising their function to overcome the toxic effects of hypoxia in HCT116 colorectal xenografts [132]. A similar methodology has been employed by investigators for regional delivery of cells including macrophages preloaded with magnetic iron particles [111], mesenchymal stem cells [133] and stem cells for the treatment of lesions [134]. Cisplatin loaded magnetic liposomes were able to overcome the drawback of low drug encapsulation efficiency by embedding magnetite nanoparticles in the liposomal membrane and the pharmacokinetics study in rats was able to sustain bioavailability in the body circulation compared to free drug [135]. Drug-containing liposomes covalently attached to magnetotactic bacteria were magnetically guided to HCT116 colorectal xenografts, with 55% of the bacteria localised to hypoxic regions [132]. Not to mention biocompatible magnetic nanoparticles (MNPs) to improve cancer

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diagnostics have been demonstrable in magnetic resonance imaging [136], radionuclide therapy [137] and hyperthermia [138-140].

The ability of MNP's to be guided externally to the target tissue could therefore circumvent the reliance on specific viral tropism or specific ligands for efficacious virotherapy, evidence for which comes from a study using ectoenzyme ALPP decorated MNPs for selectively binding cancer cells without involving specific receptor interactions or antibodies [141]. A few examples of magnetically enhancing viruses are demonstrating this potential whereby adenovirus complexed with iron oxide increased transduction efficiency in CAR-negative MCF tumours, culminating in increased cancer cell killing and intracellular replication of Ad [90, 91]. Whilst demonstrating enhanced cellular interaction with adenoviruses, these studies were performed in nude mice with intratumoural administration of virotherapy at an acute timepoints.

One of the few studies to utilise magnetic guidance of systemically administered OV's boosted HSV oncolytic activity in xenograft model of prostate cancer [142]. By disguising the OV, Sephrivir, in macrophages loaded with iron oxide NPs, investigators were able to direct them to primary and metastatic tumours using pulsed field magnetic gradients resulting in increased tumour macrophage infiltration and reduction in tumour burden. One consideration when applying magnetic gradients is their effect on cells. A large gradient (up to 1 GT/m) can significantly change the membrane potential of the cell and thus have a significant impact on not only the properties and biological functionality of cells but also cell fate [143].

In terms of nanoparticle design, the use of magnetic field gradients could allow a departure from the traditional spherical, 100nm particles which are optimal for passive circulatory interactions and margination. For example, the increased circulation times demonstrated by rod-shaped particles [18] are controversial as nanocarriers due to their poor margination but application of external forces including magnets are expected to increase margination of rod-shaped magnetic particles [20] thereby enhancing their potential to reach tumour targets via increased circulation time, increased extravasation and magnetic steering. By overcoming some of the issues concerning circulatory transport, it may allow investigators to focus nanocarrier design on the characteristics for augmentation of the latter stages, namely tissue permeation and cell entry.

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One of the limitations to investigate the interdependence of shape and size on the pharmacokinetics of magnetic nanoparticles (MNPs) has been their fabrication and production. The chemical synthesis of MNPs offers little control over uniform shape and size distribution and require coatings for biocompatibility and functionalisation. Conversely, biologically derived MNPs called magnetosomes, extracted from magnetotactic bacteria, display highly uniformed size and shape. Medical applications in oncology have so far exploited the magnetic properties of magnetosomes for magnetic resonance imaging (MRI) contrast agents [144] and magnetic hyperthermia [138]. More recently, magnetosomes have established themselves as contenders for delivery of chemotherapies. Magnetosomes loaded with DOX demonstrated comparable tumour inhibition versus DOX alone against hepatocellular carcinoma (HCC) but significantly enhanced mortality rates by reducing cardiac toxicity to DOX alone [145]. By cross-linking chemotherapies to the surface of magnetosomes Deng et al and Liu et al have generated slow releasing formulations of cytosine arabinoside (Ara-C) for acute leukemia treatment [146, 147] and co-administration of DOX with siRNA using magnetosomes protected siRNA from degradation in serum resulting in synergistic cytotoxicity *in vitro* [148]. The ability to manipulate magnetosome transport via non-invasive, external application of magnetic fields has been confirmed by Tang et al whereby human papillomavirus type E7 and Ig-Fc fragment (pSLC-E7-Fc) combined with magnetosomes generated antigen-specific cytotoxic T lymphocyte activity with resultant tumour inhibition in a murine metastatic lung model (average pulmonary metastatic tumour weight of 343.6 mg vs 58.9mg) [149].

Magnetosomes have proven themselves equally successful as existing nanocarriers which may not be surprising when we compare their structures – cubooctohedral, 30-50nm diameter surrounded by phospholipid membranes extracted from MSR-1, MC-1 and AMB-1 strains. However, a multitude of other strains have been identified that synthesise rod, bullet and cuboidal magnetosomes that could provide further opportunities to investigate the effect of shape on targeted delivery of therapies. In fact, could magnetosomes provide the ideal opportunity to optimise a carrier system that is a) not reliant on passive delivery and the EPR effect but magnetic steering for targeting and margination; b) therefore shifts the focus to particle design for permeation and cell entry; c) removes the confounding influences of composition (chemical characteristics, charge) allowing direct comparison of shape alone.

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Conclusions

Systemically delivered oncolytic viruses exist in a paradoxical scenario whereby immunosurveillance can both enhance and destroy the therapeutic potential and whereby tumour angiogenesis both facilitates viral entry but is dependent on tumour type and status. Whilst nanoparticles have demonstrated improved efficacy their design primarily focuses on optimal conditions for circulatory survival due to a reliance on the EPR effect for passive delivery. The use of active guidance systems such as MRI together with magnetic nanoparticles may allow investigators to step off the merry-go-round and select NP-OV complex designs for cell entry alone. This could mediate a complete departure from traditional nanoparticle sizes and shapes and open the door for a pan-nanocarrier for oncology.

Future prospects

The opposing design requirements within each of the three phases required for successful NP delivery indicates that there will eventually be a trade-off whereby investigators will be forced into decisions biasing one phase over another. To date this has involved intratumoural administration of OV's only; high titres for systemic administration to account for immunosurveillance; and the addition of specific targeting moieties. Unfortunately this has resulted in ineffective OV therapy for disseminated tumours; adverse effects; and precision virotherapy for single tumour types respectively. More ambitious OV-NP complexes are required (such as the design postulated in Figure 4) that encompass multiple strategies to overcome this challenge of systemic administration. Equally, the methodology required to both generate and assess NP-OV complex stability (including 3D morphology, bond strength and hydrodynamics) must be compatible within both biological and material sciences.

- **Future Perspective:** N/A
- **Executive Summary:** (bulleted summary points that illustrate the main conclusions made throughout the article. Less than 400 words).

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Introduction

- Oncolytic viruses (OV) are an attractive prospect due to their two-pronged attack mechanism: direct cell lysis and amplification of an anti-tumour immune response. Paradoxically this also results in their removal by the reticuloendothelial system when delivered systemically.
- There are three phases that OV's must navigate for successful nanoparticle delivery; circulatory longevity, tissue permeation and cellular interaction. Nanoparticles including liposomes, polymers and metallic particles are mitigating the circulatory interactions using shielding techniques and chemical modifications of their surfaces have incorporated moieties for the detection of specific tumour ligands for increased cell recognition. Despite these advances, the variable response rates evident in both primary and disseminated neoplasms suggests that efficacy relies on a multi-faceted approach.

Pharmacokinetics of nanoparticles for oncology

- It is well known that nanoparticle shape, size and composition determines their fate during each of these phases and particular characteristics have been postulated for their success. Unfortunately these characteristics are not always conducive for each phase.
- The reliance on passive delivery to the tumour target via the EPR effect has biased the current raft of nanocarriers to be optimal for navigating circulatory complications such as avoiding immune/ serum protein interactions and extravasation (spherical, ~100nm diameter, neutral charge). Therefore their limited success could be attributed to suboptimal characteristics required for the latter stages of nanoparticle delivery.

Strategies employed by nanoparticles to enhance virotherapy delivery

- The ability to actively target tumours by external guidance systems (eg. magnetic gradients) may shift the focus to characteristics required for tissue permeation and retention by circumventing the need to rely on the EPR effect. Similarly the use of environmental stimuli (eg.hypoxia) as triggers for the unveiling of specific characteristics via polymeric switches allows nanoparticles to straddle the needs of the various phases.
- The effects of shape, size, charge etc pose another problem when trying to draw comparisons due to fabrication limitations and inherent compositional differences in the various nanocarriers available.
- Ultimately, successful systemic delivery of OV's relies on how we reconcile the different needs of each phase on the merry-go-round that is nanoparticle delivery.

OR

Summary Points (Research articles & Company profiles only): 8–10 bullet point sentences highlighting the key points of the article.

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- **Figure legends**

Figure 1. The nanocarrier design merry-go-round. Optimal nanocarrier characteristics for each phase of systemic delivery of cancer therapies. AR = aspect ratio.

Figure 2. Currently used nanocarriers demonstrating significant similarities; spherical morphology, synthetic or biological phospholipid coating and overlapping diameters.

Figure 3. Factors controlling pharmacokinetics and biodistribution of nanoparticles (NP). Size: Large NPs (1) will not be able to enter the tumour through leaky vasculature and demonstrate increased blood protein deposition as a result of increased surface area resulting in rapid clearance. Small NPs show high permeation rate (2) but also are rapidly cleared from the tumour by RES (3) leading to poor accumulation. Shape of NPs (4) affects tumour cell internalisation and determines interaction with RES, PK and tumour retention as a result of surface curvature and altered hydrodynamic behaviour. Aggregates can cause different organ distribution. **Surface characteristics:** Polymer coated NPs may shield NPs from neutralising Abs and RES but repeated administration can induce anti-PEG antibodies (5). Negatively charged NPs exhibit strong RES uptake whilst cationic NPs induce serum protein aggregation exhibit increased uptake by cells (6). Neutral NPs can travel up to three times more distance than charged analogues (7), distribute more evenly and exhibit least RES interaction for the longest circulation. PEGylation can shield charge effects (8) preventing opsonisation and can be shed after tumour extravasation to expose cationic particles that can interact with target cells (9). **Regime:** Multiple treatments could either increase generation of neutralising antibodies (10) to NPs resulting in reduced efficacy or suppress RES activity during the first dose but reduce clearance and increase toxicity of subsequent doses (11). Distribution of NPs is dependent also on exposure route (12).

Figure 4. Current methods for virotherapeutic targeting of tumours and how to manipulate these further to create the ideal NP-OV complex.

- **Table Legends**

Table 1: Multiple oncolytic pathways of oncolytic viruses

Table 1: Multiple oncolytic pathways of oncolytic viruses

Advantage	Description	Virus	Ref

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Direct Oncolysis	Consequence of viral replication and virion release.	HSV, VV, VSV	[150]
	Cytotoxic proteins synthesised by the virus e.g. induction of apoptosis by adenovirus E4-ORF3 protein expression.	Ad	[151]
Reprogramming the tumour microenvironment	Release of DAMPs and TAAs into bloodstream thereby switching an immunogenically "cold" tumour (low abundance of tumour antigens, tumour infiltrating lymphocytes and an immunosuppressive tumour environment) to a 'hot' immunogenic tumour.	HSV	[152, 153]
	Activation of innate and adaptive anti-cancer immune responses, thereby inducing immunogenic cell death (ICD) in cancer cells.	Ad, MV	[154-158]
	Mediate the killing of uninfected cancer cells by destruction of tumour blood vessels.	VSV, VV	[159-161]
Enhance anti-tumour effects	Amplification of therapy overtime due to virion release and immune interaction in comparison to classical drug pharmacokinetics which decrease with time.	Ad	[162]
Inhibit relapse and metastasis	Incorporation of transgenes (eg. GM-CSF, IFNα/β, IL-12, CCL5).	HSV, Ad, VSV, VV	[163]

DAMPs = damage-associated molecular patterns, TAAs = tumour associated antigens, VSV = vesicular stomatitis virus, VV = vaccinia virus, Ad = adenovirus, MV = measles virus

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- **Reference annotations**: authors should highlight 6–8 references that are of particular significance to the subject under discussion as **"* of interest"** or **"** of considerable interest"**, and provide a brief (1–2 line) synopsis.

Figure 1

175x152mm (150 x 150 DPI)

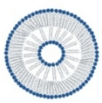
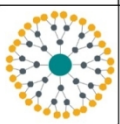
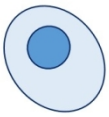
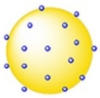
Lipid nanocarriers		Polymeric nanocarriers		Organic nanocarriers	Metal nanocarriers		
Liposome	Micelles	Polymer	Dendrimer	Cells	Gold nanoparticle	Magnetosome	Iron oxide nanoparticle
							
25-2500nm	2-20nm	10-1000nm	1-100nm	25000nm	5-400nm	30-120nm	1-100nm

Figure 2

266x75mm (150 x 150 DPI)

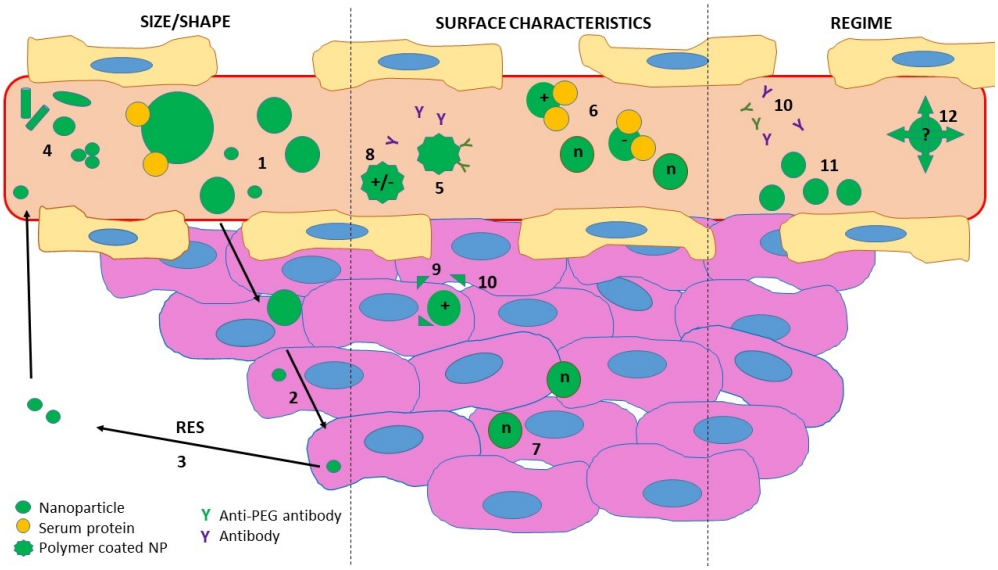


Figure 3

338x190mm (96 x 96 DPI)

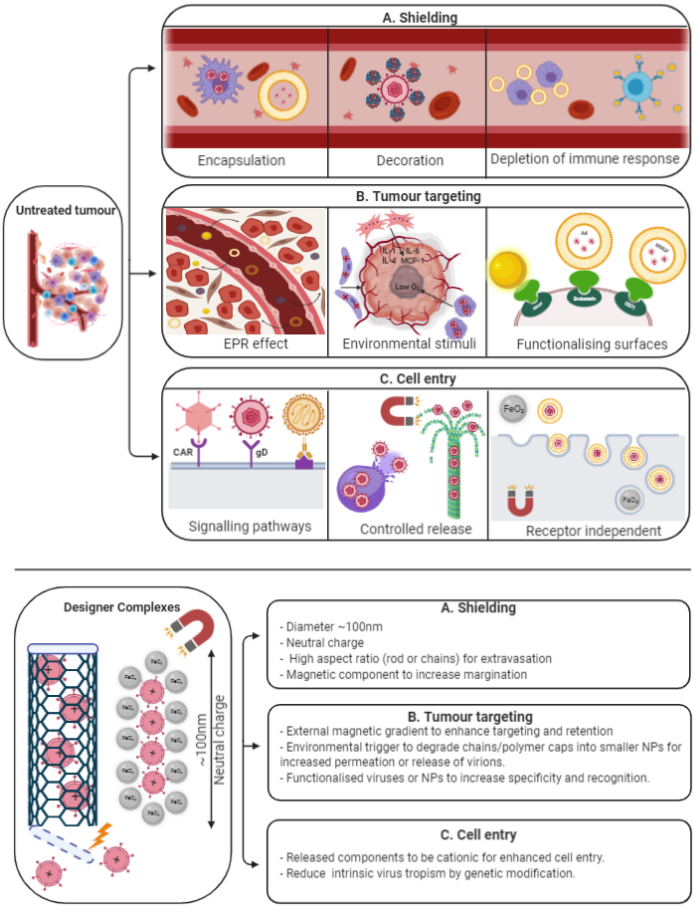


Figure 4