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A Cure for Cancer? The Therapeutic Potential of Combining Oncolytic Viruses and Chimeric Antigenic Receptor T-Cell Therapy

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SUMMARY Cancer continues to be a devastating diagnosis. Fortunately, active research has developed and refined two cancer therapies: oncolytic virotherapy and the novel chimeric antigen receptor (CAR) T-cell therapy. The former utilizes oncolytic viruses (OVs) that preferentially infect and kill cancer cells over healthy, non-cancerous tissues. The latter isolates the patient's T-cells, uses a disarmed virus to insert genes expressing genetically engineered receptors, known as chimeric antigen receptors, which target tumor-associated antigens on malignant cells. Despite several clinical successes with both therapies, limitations exist which prevent their medicinal potentials from being achieved. Oncolytic viruses are often completely sequestered or neutralized due to human physiology and pre-existing adaptive immunity. CAR T-cells seldom enter solid tumors due to an insufficient number of chemokines secreted by the tumor, or because tumor-associated antigens are rarely released. Even if entry occurs, the tumor microenvironment is often in an immunosuppressed state that greatly diminishes T-cells from functioning. Remarkably, it has been previously reported that the administration of oncolytic viruses into patients, even with sequestration, can result in the release of neoantigens from tumors, which may promote entry of CAR T-cells into solid tumors. Furthermore, oncolytic viruses have been shown to reverse the immunosuppressive environment of certain tumors. With this, CAR T-cells may be able to perform their critical role of orchestrating the immune system to kill malignancies. For the millions of individuals who will be diagnosed with cancer, it is critical that researchers investigate the therapeutic potential of combining oncolytic virotherapy and chimeric antigen receptor T-cell therapy.

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INTRODUCTION

Cancer's ubiquity continues to devastate the lives of patients, their families and friends, and healthcare systems around the world. Furthermore, Canada's aging population presents a major risk for a projected increase in cancer incidence – since cancer is typically

associated with aging [1]. It is estimated that by 2036, 25% of the Canadian population will be composed of adults 65 years of age and older [2]. Adults in this age group are 11 times more likely to develop cancer than those under 65 years old [3]. With an anticipated increase of cancer incidence, it is therefore critical that discovery, testing, and refinement of novel treatments continue.

An emerging cancer treatment modality is oncolytic virotherapy. This treatment utilizes natural or engineered oncolytic viruses (OVs) that have a selective, but not nonexclusive, tropism for tumors [4]. It has been previously assumed that OVs' primary anti-cancer effect was their oncolytic capability [5]. However, oncolysis only plays a minor role in cancer therapy [5]. Instead, OVs convert tumor microenvironments (TME) into "vaccine factories" [5]. These "factories" induce the release of immunogenic small molecules and protein mediators, such as type I interferon (IFN), adenosine triphosphate (ATP), uric acid, and high-mobility group box 1 (HMGB1) [5]. With the release of the signals, comes enhanced tumour-associated antigen presentation, increased T-cell and natural killer cell trafficking into the TME, and enhanced effector function [5].

Chimeric antigen receptor (CAR) T-cell therapy is another promising anti-cancer remedy. This therapy is generally catered for refractory hematological malignancies and immunogenic cancers, such as melanoma and renal cell carcinoma. Currently, the Food and Drug Administration (FDA) has approved two CAR T-cell therapies for acute lymphoblastic leukemia and non-Hodgkin's lymphoma. CARs are recombinant cell surface fusion proteins that contain both extracellular domains designed to bind to tumor-specific or tumor-associated cell surface antigens, and intracellular signalling domains [6,7]. These intracellular activating and co-stimulatory domains mimic physiological T-cell receptors, but act in a MHC-independent manner [5]. Once antigens are bound to the extracellular domain, T-cell activating signals occur [6,7]. CARs can also target carbohydrate or glycolipid structures in addition to proteins, unlike T-cell receptors [6].

RESEARCH QUESTIONS

Cancer is a devastating disease that touches all lives in one way or another. New treatment options such as oncolytic virotherapy and CAR T-cell therapy for cancer and refractory malignancies exist and are being actively pursued, respectively. Despite their individual successes, limitations currently exist for both therapies. Certain limitations of each therapy have been theoretically resolved by complementing one therapy with the other. In order to determine whether combining these two therapies could improve cancer prognosis, this review focuses on the following research questions:

1. What are the current limitations for oncolytic virotherapy and chimeric antigen receptor T-cell therapy?
2. How would combining oncolytic virotherapy and chimeric antigen receptor T-cell therapy minimize their respective limitations and thus improve cancer prognosis?
3. What are the potential risks of combining oncolytic virotherapy and chimeric antigen receptor T-cell therapy?

PROJECT NARRATIVE

What are the current limitations for oncolytic virotherapy and chimeric antigen receptor T-cell therapy?

Oncolytic virotherapy and CAR T-cell therapy is an actively pursued area of research in hopes of decreasing cancer-related mortalities. Despite recent advances in their fields, limitations exist which prevent these treatments from achieving their therapeutic potential. OVs are administered to patients systemically or via intra-tumoral injection [5]. For many cancers, tumors are not often easily accessible, or are metastatic. Therefore, systemic delivery of OVs are often necessary for any therapeutic effect. However, a predominant barrier to effective systemic oncolytic virotherapy administration is patient host defences [8].

Leukocytes, the complement system, antibodies, and antiviral cytokines may all limit the functioning and delivery of OVs into their target cells [9]. OVs are further limited by pre-existing immunity in the patient. Immunity may be developed by prior immunization, or simply accidental exposure due to the ubiquitous nature of the virus [8]. The vaccinia virus, a heavily researched oncolytic virus was used for the worldwide eradication of smallpox [8].

Therefore, many individuals who undergo oncolytic virotherapy have immunity to this particular vaccinia OV. In addition, certain OVs, such as Reovirus, are globally abundant. Consequently, many patients will have immunity to viruses due to immunizations, or by random exposure, and thus prevent oncolytic virotherapy from succeeding [10, 11].

Sequestration by the lung, liver, and spleen will also decrease systemically administered OVs' ability to infect tumors throughout the body [8]. A study investigating oncolytic adenovirus and its tendency to be sequestered by the liver, for example, showed that approximately 90% of the intravenous injected adenovirus was taken up by the liver [9]. They concluded that the rapid uptake by the liver lead to hepatotoxicity, reduced virus uptake by the target tumor tissue, and ultimately, a decreased therapeutic efficacy [9]. A summary of these obstacles is presented in Figure 1.

Unlike oncolytic virotherapy which has shown efficacy against certain solid tumors [12], CAR T-cell therapy has only shown clinical benefit against hematological cancers [13, 14, 15, 16]. This can possibly be explained by CAR T-cells' poor ability to enter solid TMEs [17]. For successful entry, a sufficient number of T-cell attracting chemokines secreted by tumors are needed [17]. Moreover, tumor-specific or tumor-associated antigens recognized by CARs, which are also required for entry, have been difficult to identify on solid tumors [17]. In addition to entry, tumors often contain immune inhibitory pathways which help suppress attack by T-cells [18].

How would combining oncolytic virotherapy and chimeric antigen receptor T-cell therapy minimize their individual limitations and thus improve cancer prognosis?

Oncolytic virotherapy and CAR T-cell therapy have amassed much excitement from researchers, clinicians and patients. A possible novel cancer therapy which may resolve their individual limitations, is to successively administer OVs and CAR T-cells into patients. A major limitation of CAR T-cell therapy on solid tumors is the lack of ideal TAAs [17]. With

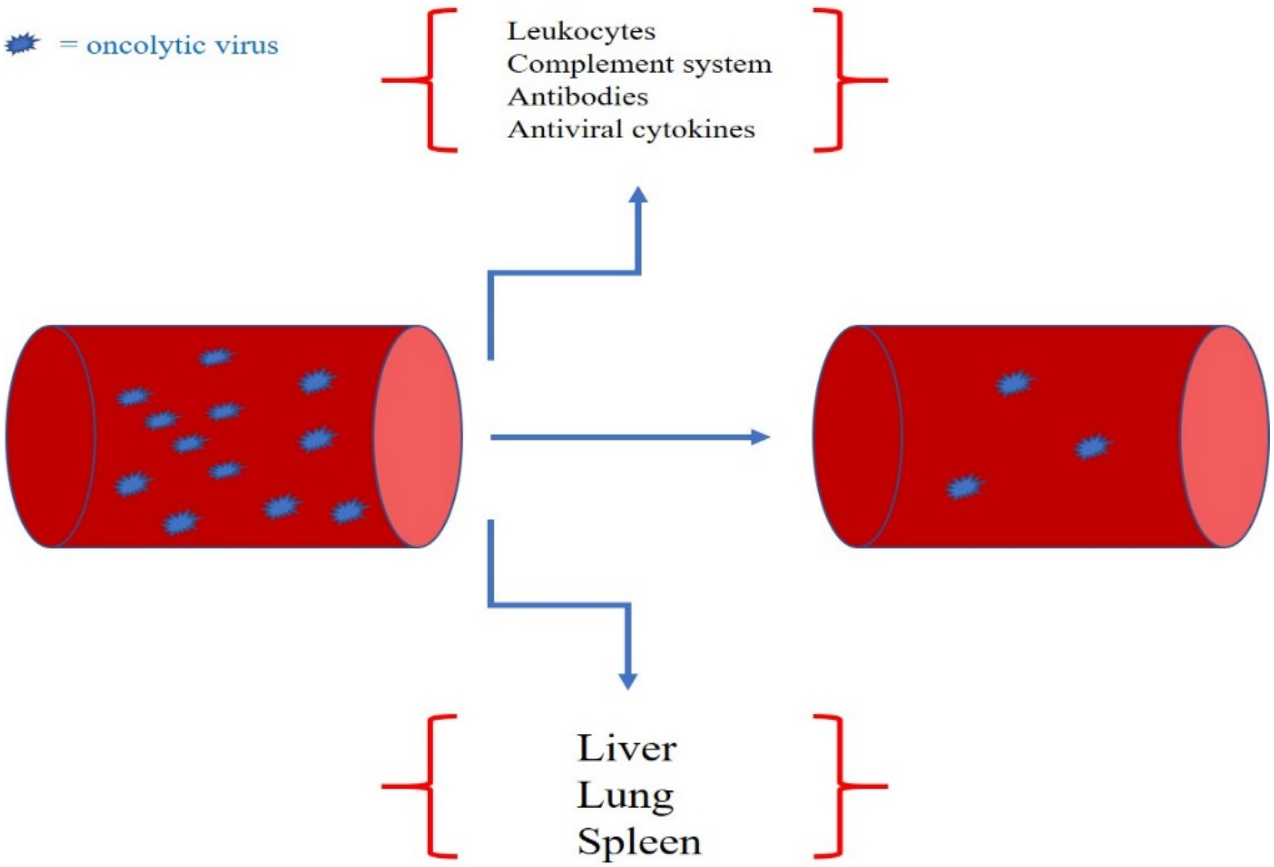

FIG. 1 A brief summary of potential obstacles preventing successful systemic administration of oncolytic viruses into a patient.

an initial dose of OVs, despite sequestration by host defences or by pre-existing immunity, the oncolytic properties of OVs may cause the release of neoantigens from tumors [19]. The released neoantigens will have not yet been recognized by the patient host and will result in an immune response, thus enhancing CAR T-cells' entry into tumors.

With a potential surge of CAR T-cells entering into tumors due to an increased release of neoantigens, it has been reported that the immunosuppressive TME inhibits T-cells' anti-tumor effects [20]. This is done via an enhanced expression of checkpoint inhibitors, differentiation of regulatory T-cells [21], myeloid-derived suppressor cells [22], tumor-associated macrophages [23, 24], and mesenchymal stem cells [25, 26]. Interestingly, OVs may be able to transform the TME into an immunogenic environment. When cells are infected with viruses, type I IFNs are often released to produce an antiviral state. Although tumors may become resistant to the OVs, the released type I IFNs will up-regulate MHC I on all cells, increase macrophage and natural killer cell activation [27], and promote the activation and survival of both $CD4^+$ and $CD8^+$ T-cells [28, 29]. Therefore, OVs could be used to prime the reversal of the immunosuppressed TME and enhance the anti-cancer effects of CAR T-cells.

In addition to the regular anti-viral cellular response to viruses, it may be additionally beneficial to use recombinant OVs encoding transgenes. These genes could express various interleukins, IFNs, and apoptosis-inducing ligands [30, 31] which could further promote an immunogenic state in the TME. It has even been reported that several of these gene products have cytotoxic effects on neighbouring uninfected cancer cells [32].

Treating cancer is often a patient-by-patient approach. All cancers and patients are different, so efficacy of CAR T-cell entry into tumors should also theoretically differ. For patients whose cancer adequately permits CAR T-cell entry, it may be advantageous to initiate treatment with CAR T-cells encoding OV DNA. By doing so, fully infectious genomes can be protected from neutralizing antibodies, protease degradation, or complement inactivation [33]. Furthermore, the patient's tendency to sequester OVs will be greatly decreased given that free OVs will not be present in the circulation.

What are the potential risks of combining oncolytic virotherapy and chimeric antigen receptor T-cell therapy?

The therapeutic effects of combining OVs and CAR T-cells appear to be additive, if not synergistic. While these individual therapies have side effects and other barriers, this novel combinatory therapy will also have additional obstacles that must be considered.

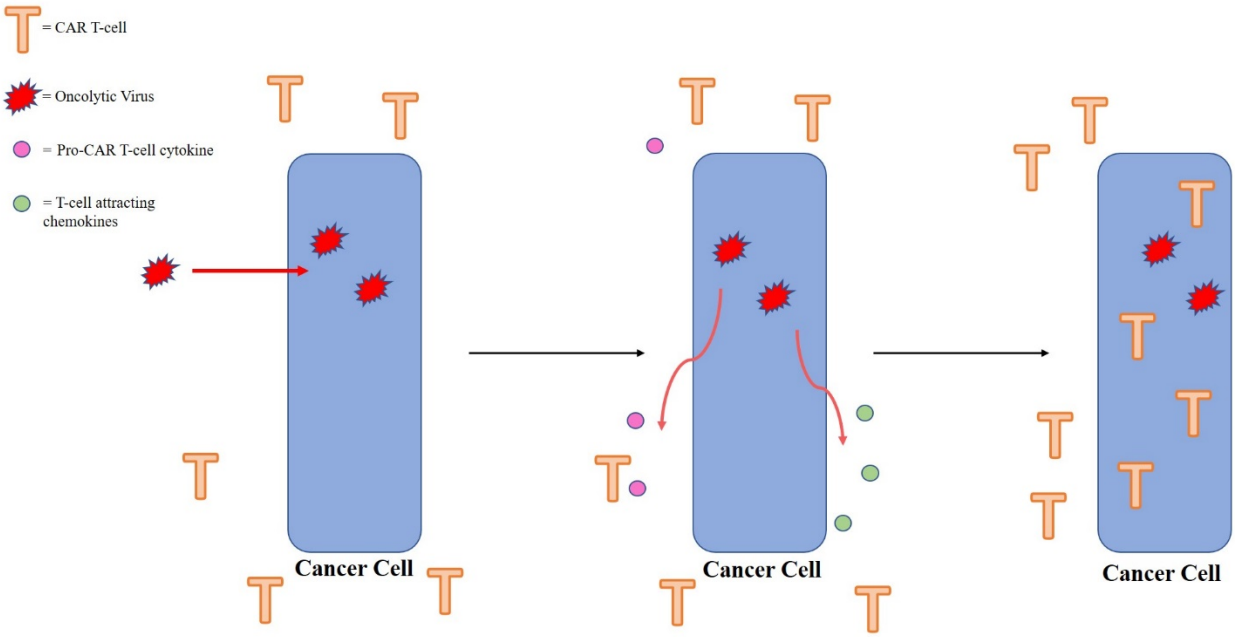

FIG. 2 Oncolytic virus encoding transgenes expressing and releasing pro-CAR T-cell cytokines and T-cell attracting chemokines, resulting in increased chimeric antigen receptor T-cell entry into cancerous cells.

The first obvious limitation of combining oncolytic virotherapy with CAR T-cell therapy is cost. The price of advanced melanoma treatment with Talimogene laherparepvec (T-VEC), a 2015 FDA approved OV, for example, approximately costs each patient $65,000 USD for a full treatment [34]. The more recently FDA approved (August 2017) CAR T-cell therapy, Kymriah, costs each patient roughly $475,000 USD [35]. Therefore, it is reasonable to predict that a treatment with OVs and CAR T-cells combined may amount to >$500,000 USD. Despite this vast sum, there are currently only two FDA approved CAR T-cell therapies in the USA. As research progresses and additional CAR T-cell therapies are available to the public, the increased competition amongst pharmaceutical companies will likely result in lower CAR T-cell therapy costs. Precise estimations of lowered costs are difficult to predict. While considering the various fruitful ways of combining OVs and CAR T-cells, one must also understand that the therapies can antagonize each other, if not combined carefully. Chimeric antigen receptor T-cells can elicit strong anti-viral conditions. If too many cytokines are released by the CAR T-cells, the oncolytic and "vaccine-developing" properties of OVs may be suppressed, if not eliminated. Likewise, if OVs primarily destroy cancer cells via apoptosis rather than immunogenic cell death, CAR T-cells will likely have little effect on cancer cells [5].

Chimeric antigen receptor T-cell therapy is known to have a serious potential side effect, where an excessive number of cytokines are released, termed cytokine release syndrome [36, 37]. Although potential toxicity from CAR T-cells can be better estimated with non-human primate testing [38], the addition of OVs in cancer therapy will likely also result in additional cytokine release. Therefore, it is critical to carefully implement OVs and CAR T-cells that result in a sustainable and non-life-threatening amount of cytokine release.

SUMMARY AND CONCLUSION

Cancer's immense negative influence on the lives of patients cannot be overstated. In Canada alone, nearly one in two Canadians will be diagnosed with cancer in their lifetimes, and one in four will die [39]. As a result, novel cancer therapies are rapidly emerging, and present treatments are currently being refined. However, despite active research, therapies such as oncolytic virotherapy and CAR T-cell therapy, have proven to be inefficacious for certain cancers or patient populations. This paper, however, has reviewed and proposed certain complementary effects from combining these therapies that may result in potential therapeutic synergy.

The combinatory effects of OVs and CAR T-cells have not been thoroughly investigated, therefore questions remain. For instance, is there currently an ideal OV to use when combined with CAR T-cells? It could be argued that one does not currently exist, or that it depends on the patient's cancer and adaptive immunity. However, one possible OV to investigate is the vaccinia virus. For one, genetically engineered vaccinia viruses have entered clinical trials, and much is known about this virus [40]. Secondly, if sequestration or patient immunity of OVs poses an obstacle for tumor entry, the extracellular enveloped vaccinia virus has evolved in a manner that renders the virus relatively resistant to complement- and antibody-mediated neutralization [40]. Furthermore, the genome of vaccinia is large, and enables the insertion of many foreign genes. For tumors that have an immunosuppressive TME and CAR T-cells that have difficulty entering solid tumors, transgenes expressing pro-T-cell cytokines and T-cell attracting chemokines are imperative. A model of this occurring is presented in Figure 2.

Theoretical arguments exist (and have been stated in this paper) that support beginning treatment with OVs, then administering CAR T-cells. However, reasons for reversing this order also exist. For instance, for metastatic cancer present in the brain, cell carriers such as CAR T-cells, may need to be used to carry OVs across the blood-brain barrier [41]. Furthermore, it is also probable that pre-existing adaptive immunity and sequestration of OVs in *certain* patients will result in an ineffective concentration of OVs, rendering its pro-CAR T-cell therapy properties inadequate. For these reasons, it is important to investigate a combination therapy beginning with CAR T-cell therapy and not OVs, and also CAR T-cells encoding complete viral genomes.

Combining OVs and CAR T-cells for cancer therapy will have its own limitations and challenges. Given this, this combinatory therapy may not be feasible. However, entertaining

the idea may encourage researchers to not only pursue the discovery of novel cancer treatments but also investigate the therapeutic potential of combining already existing cancer therapies. The benefits of combining these treatments may be exponentially enhanced and could ultimately play a major role in the battle against cancer.

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