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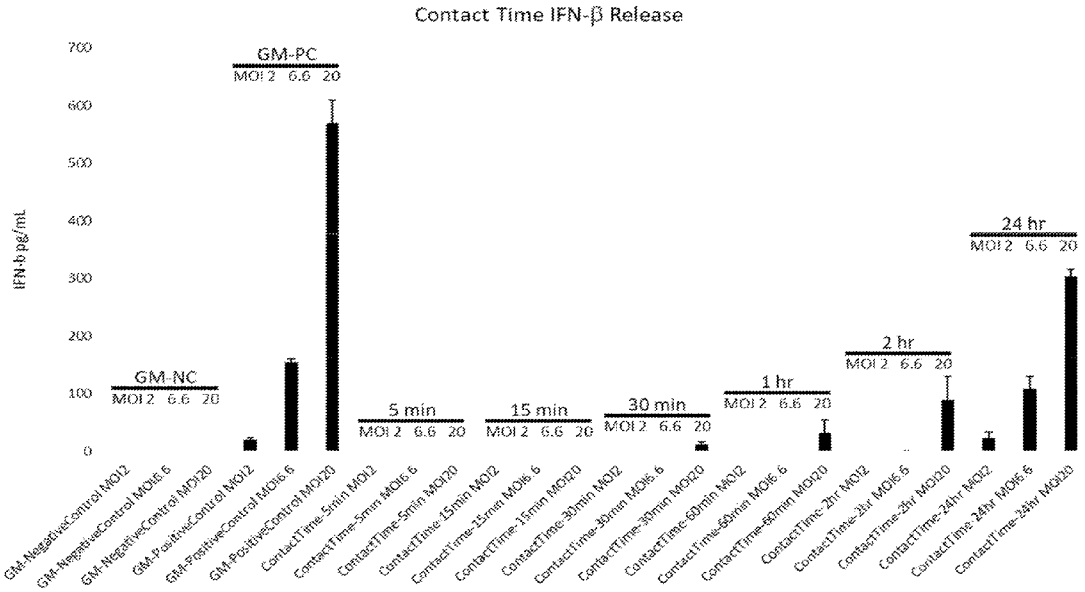

FIG. 3C

(57) Abstract: The present invention provides improved methods to treat bladder cancer, including non-muscle invasive bladder cancer (NMIBC), in a human subject comprising intravesical instillation administration to the patient of a high dose of a chimeric poliovirus construct comprising a Sabin type I strain of poliovirus with a human rhinovirus 2 (HRV2) internal ribosome entry site (IRES) in the poliovirus 5' untranslated region between the poliovirus cloverleaf and the poliovirus open reading frame (a "chimeric poliovirus"). As provided herein, the chimeric poliovirus is administered by intravesical instillation in a particular treatment regime comprising an induction phase and a maintenance phase.

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METHODS FOR TREATING BLADDER CANCERS BY INTRAVESICAL INSTILLATION OF A CHIMERIC POLIOVIRUS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/301,008 filed January 19, 2022, U.S. Provisional Application No. 63/310,008 filed February 14, 2022, U.S. Provisional Application No. 63/317,851 filed March 8, 2022, and U.S. Provisional Application No. 63/350,314 filed June 8, 2022. The entirety of each of these applications is hereby incorporated by reference herein for all purposes

INCORPORATION BY REFERENCE

The contents of the text file named "21081-033WO1_ST26.xml" which was created on January 19, 2023, and is 9.1 KB in size, are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to improved methods for treating bladder cancer, including non-muscle invasive bladder cancer, (NMIBC) by altering the tumor microenvironment to promote a pro-inflammatory immune response to the tumor via specifically timed intravesical instillation administrations of a chimeric poliovirus.

BACKGROUND OF THE INVENTION

Cancer of the bladder, also known as urological cancer and urinary bladder cancer, is the 10th most common cancer worldwide and 13th most deadly, with the incidence rate rising globally (Saginala et al. Med Sci. 8(1):15(2020)). Within the United States, it was estimated that approximately 81,000 patients would be diagnosed with, and 18,000 patients would die from, bladder cancer in 2020, with around 75% of the cases occurring in men with an average diagnosis age of 73 years (Richters et al. World J Urol. 38(8):1895-1904(2020)). Up to 95% of bladder cancer cases arise from urothelial cells that line the bladder and urinary tract (termed urothelial carcinoma or transitional cell carcinoma), with the strongest risk factors being tobacco smoking (~65% of cases) or occupational or environmental hazards (20% of cases). The remaining cases are attributed to squamous cell bladder cancer (due to the protozoan infection schistosomiasis) or

rare subtypes such as adenocarcinoma, sarcoma, and metastases to the bladder (Saginala et al. Med Sci. 8(1):15(2020)). The average 5-year survival rate for bladder cancer in the United States is 77.1%, ranging from 95.8% for low-risk localized disease to 4.6% for metastatic disease (Surveillance, Epidemiology, and End Results (SEER) Program. National Cancer Institute. SEER* Explorer). Fortunately, the majority of patients (~80%) are diagnosed with localized disease (non-muscle invasive bladder cancer ([NMIBC], stages Ta, T1) which carries a better prognosis than more advanced stages where the cancer has invaded the muscle layer of the bladder (muscle invasive bladder cancer [MIBC]) and/or metastasized to the regional lymph nodes or beyond (American Cancer Society. Bladder Cancer. (2020)).

Non-muscle invasive bladder cancer (NMIBC) comprises noninvasive papillary carcinomas (Ta; 48%), submucosal invasive tumors (T1; 27%), carcinoma in situ (CIS; 2%) or some combination of these types (Boustead et al. BJU Int. 113(6):924-30(2014); Chang et al. J Urol. 196(4):1021-9(2016)); and may be categorized as low-, intermediate-, or high risk of experiencing recurrence and/or progression of bladder cancer (Table 1). These risk categories are intended to guide clinicians in treatment and surveillance decisions based on disease prognosis.

Table 1. AUA Risk Stratification for Bladder Cancer Recurrence and/or Progression

Low Risk	Intermediate Risk	High Risk
Low grade solitary Ta ≤ 3cm	Low grade Ta that recurs within 1 year	High grade T1
Papillary urothelial neoplasm of low malignant potential	Solitary low grade Ta > 3cm	Any recurrent high grade Ta
	Multifocal low grade Ta	High grade Ta > 3cm or multifocal
	High grade Ta ≤ 3cm	Any carcinoma in situ
	Low grade T1	Any BCG failure in high grade patient
		Any variant histology
		Any lymphovascular invasion
		Any high grade prostatic urothelial involvement

Transurethral resection of all visible lesions is a standard treatment for NMIBC (Babjuk et al. Eur Urol. 71(3):447-61(2016)) but is accompanied with an exceedingly high tumor recurrence rate ranging from 50% to 70% as well as a high tumor progression rate into muscle-invasive bladder cancer type between 10% and 20% over a period of 2 to 5 years (Chen et al. Chin Med J. 123(23):3422-6(2010)). Thus, guidelines recommend chemotherapy or immunotherapy in the management of NMIBC to reduce these risks of recurrence and progression (Babjuk et al. Eur Urol. 71(3):447-61(2016)).

Immunotherapies include the intravesical administration of BCG, a live attenuated strain of *Mycobacterium bovis*. The recommended standard of care for patients with NMIBC involves transurethral resection of bladder tumor (TURBT) followed by intravesical chemotherapy or Bacillus Calmette-Guérin (BCG) non-specific immunotherapy depending on the patient's risk group (Flaig et al. J Natl Compr Canc Netw. 18(3):329-54(2020); Chang et al. J Urol. 196(4):1021-9(2016); Babjuk et al. Eur Urol. 76(5):639-57(2019)). Low-risk tumors are conventionally managed with single dose intravesical chemotherapy while high-risk tumors are managed with adjuvant intravesical BCG (Morales et al. J Urol. 116:180-3(1976)). Intermediate-risk tumors may be managed with either intravesical chemotherapy or BCG.

BCG immunotherapy may decrease the frequency of—and delay the time to—cancer recurrence and progression in patients with NMIBC (Babjuk et al. Eur Urol. 71(3):447-61(2016); Braasch et al. BJU Int. 102:1254-64(2008)). There remains, however, a 50% failure rate with patients receiving BCG (Packiam et al. Urol Oncol Semin Orig Investig. 36:440-7(2018)). Patients who do not respond to BCG therapy are classified into four refractory subgroups, including BCG refractory, BCG relapsing, BCG unresponsive, and BCG intolerance. Studies indicate that patients who experience no benefits at 6-months of BCG therapy are at a higher risk of disease recurrence and progression compared with patients who have not been administered BCG (Table 1) (Shirakawa et al. BJU Int. 110:E216(2012); Herr et al. Urol Oncol. 33:108(2015)). Treatment options for BCG-refractory patients are limited and patients are often recommended to undergo cystectomy (bladder reomoval). Intravesical BCG therapy is also associated with significant risk for a patient to experience an adverse event, with approximately 70% of all NMIBC patients reporting local or systemic side effects (Brausi et al. Eur Urol. 65(1):69-76(2014)). Moreover, 33% of all NMIBC patients experience serious local and systemic side effects due to BCG

infection (Bassi et al. Sug Oncol. 11(1-2):77-83(2002); Fuge et al. Res Rep Urol. 7:65-79(2015)). Reported side effects most commonly include cystitis, dysuria, and haematuria (Brausi et al. Eur Urol. 65(1):69-76(2014)). Some lethal side effects, although uncommon, have been associated with intravesical BCG therapy, including death occurring from systemic BCG infection and sepsis (Rawls et al. J Urol. 144:1328-1330(1990); Lamm et al. J Urol. 147:596-600(1992)). The frequency and severity of side effects associated with BCG therapy compel the development of novel therapeutics with superior safety profiles.

Furthermore, there is a worldwide shortage of BCG due to the notoriously lengthy (3-4 months) and costly manufacturing process which possibly disincentivizes the production of the drug. Production shortages have historically contributed to a substantial percentage of patients failing to reach completion of the standard BCG treatment regimen, which totals 27 total administrations over several years (Lenis et al. Clin Genitourin Cancer. 15:e25-e31(2017)). Despite the bona fide survival benefits associated with BCG, only 16% of patients in the pivotal SWOG 8507 clinical trial which helped to establish the administration schedule of BCG completed the full regimen (Lamm et al. J Urol. 163(4):1124-9(2000)), and moreover, the rate of real-world compliance is expected to be much lower. Combining the concerns/limitations with BCG immunotherapy and a potential worldwide shortage of BCG (Fankhauser et al. Curr Opin Urol. 30(3):365-9(2020)), there is an urgent need to develop novel therapies for this disease.

Treatment regimens comprising the intravesical administration of chemotherapy agent(s) either in combination with BCG therapy or in patients who have failed BCG therapy have shown some benefit. The chemotherapy agents currently being tested in bladder cancer patients comprise platinum-based drugs including cisplatin and carboplatin and non-platinum-based drugs including mitomycin C, doxorubicin, epirubicin, gemcitabine, docetaxel, methotrexate, vinblastine, and cabazitaxel. A standard of care in NMIBC has been a single post-TURBT intravesical instillation of mytomicin C which has been demonstrated to lower recurrence rate depending on the bladder cancer subtype (Sylvester et al. J Urol. 171:2186-90(2004)). The efficacy of mitomycin C administration in combination with BCG is unclear, however. Recently, much progress has been made in testing the efficacy of various other chemotherapeutic agents in the treatment of bladder cancer. In a recent trial, gemcitabine prevented progression to muscle-invasive bladder cancer, although gemcitabine in combination with another chemotherapy agent further improved anti-

tumor effects observed (Valaer et al. Curr Urol Rep. 17:38(2017)). The standard of care of patients with muscle-invasive bladder cancer (MIBC) is neoadjuvant chemotherapy comprising platinum-based agents (e.g., cisplatin) followed by radical cystectomy which is associated with response rates near 50% (Petrelli et al. Eur Urol. 65:350-7(2014); von der Maase et al. J Clin Oncol. 23:4602-8(2005)). Up to 40% of these patients experience relapse following cystectomy and eventually succumb to the disease (Bellmunt et al. Semin Oncol. 39:598-607(2012)). There remain substantial challenges associated with chemotherapeutic treatment of bladder cancer patients however, with overtreatment and resulting toxicity chiefly among them (Taber et al. Nat Commun. 11(1):4858(2020)).

Overall, these data indicate that there is still significant unmet medical need for patients with all stages of bladder cancer, including NMIBC, where the goal of improving therapy ranges from decreasing the frequency of/need for repeat surgical intervention and preserving the bladder/avoiding cystectomy, to prolonging survival without adding additional toxicity. Taken together, there is significant interest in the development of novel immunotherapeutic agents that can safely increase the anti-tumor response for a substantial proportion of bladder cancer patients that are unable to benefit from current FDA approved therapies.

SUMMARY OF THE INVENTION

The present invention provides improved methods to treat bladder cancer, including non-muscle invasive bladder cancer (NMIBC), in a human subject comprising intravesical instillation administration to the patient of a high dose of a chimeric poliovirus construct comprising a Sabin type I strain of poliovirus with a human rhinovirus 2 (HRV2) internal ribosome entry site (IRES) in the poliovirus 5′ untranslated region between the poliovirus cloverleaf and the poliovirus open reading frame (a "chimeric poliovirus") in the bladder. As provided herein, the chimeric poliovirus is administered by intravesical instillation in a particular treatment regime comprising an induction phase and a maintenance phase. In some embodiments, the chimeric poliovirus is lerapolturev (also known as PVSRIPO).

Lerapolturev is an oncolytic virus capable of direct anti-tumor effects through the cytotoxic infection of tumor cells. Unlike other oncolytic viruses, however, lerapolturev is also capable of non-lethal infection of many immune effector cells, including tumor associated macrophages

(TAMs) and dendritic cells (DCs), which express the CD155 poliovirus receptor. The non-lethal infection of immune effector cells leads to activation of secondary immune responses via triggering of innate interferon (IFN) pathways against tumor neoantigens. Upregulation of Type 1 IFN signaling in the TME leads to the generation of a systemic cytotoxic T lymphocyte (CTL) effector anti-tumor response. In comparison, infection of immune effector cells with other oncolytic viruses (e.g., herpes simplex virus I) impedes the ability of DCs to mount an antiviral immunomodulatory response.

As shown herein, targeted TME cellular infection of bladder cancers such as NMIBC via intravesical instillation using a chimeric poliovirus, for example lerapolturev, is not adversely affected by chemicals that model biofluids (e.g., saliva, urine) or supplemental agents (e.g., detergent) (see, e.g., FIG. 1A-E), rendering delivery via intravesical instillation within the bladder a promising therapeutic approach. Cells infected with lerapolturev are capable of mounting a robust Type 1 interferon beta (IFN-β) immunomodulatory response across a wide variety of environmental conditions (see, e.g., FIG. 2A-2E), indicating the capability of generating a systemic cytotoxic T lymphocyte (CTL) effector anti-tumor response (see Brown et al. Science Translational Medicine. 9(408)(2017)) and potent anti-tumor potency in the host subject. Remarkably, only a short period of contact time between the chimeric poliovirus and targeted TME cells is needed to achieve infectivity and trigger an immunomodulatory response, with responses observed with contact times as low as 30 minutes (see, e.g., FIG. 3A-3C), thus making delivery via intravesical instillation ideal.

As provided herein, a specifically-timed administration schedule of a chimeric poliovirus by intravesical instillation may provide enhanced efficacy and reduced tumor recurrence rates in subjects with bladder cancer. For example, a chimeric poliovirus is administered to the subject at specifically timed intervals for the initiation of an immune effector cell response during an induction phase. Following the induction phase, the subject can be further administered the chimeric poliovirus at specific times to maintain or further enhance the immune response to the bladder cancer during a maintenance phase. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, the improved methods herein are effective in subjects who were previously administered anti-cancer therapies but have developed an acquired resistance to such treatment, had a primary resistance to such treatment, have progressed on such

treatment, or had a cancer recurrence during or following such treatment. In some embodiments, the improved methods herein provide reduced tumor recurrence rates in subjects. In some embodiments, the improved methods herein provide reduced tumor progression rates.

In one aspect, an effective amount of a chimeric poliovirus is administered to a subject with bladder cancer during an induction phase, wherein the induction phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of the chimeric poliovirus once per week for 6 weeks, and wherein the chimeric poliovirus is administered via intravesical instillation. In some embodiments, the induction phase comprises 2 or more treatment cycles. In some embodiments, the induction phase comprises 2, 3, 4, 5, or more than 5 treatment cycles. In some embodiments, the induction phase comprises 2 treatment cycles. In some embodiments, the induction phase comprises 3 treatment cycles. In some embodiments, the induction cycle is repeated if no objective response rate (ORR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no complete response (CR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no partial response (PR) is exhibited by the patient. In some embodiments, the subject is administered the chimeric poliovirus in an induction phase only. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, the method is administered until disease progression or death. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In one aspect, an effective amount of a chimeric poliovirus is administered to a subject with bladder cancer during a maintenance phase, wherein the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of the chimeric poliovirus once a week by intravesical instillation, wherein each treatment cycle lasts 1-week, 2-weeks, 3-weeks, 4-weeks, or 6 weeks, and wherein the initiation of each treatment cycle is 4 weeks apart, 6 weeks apart, 8 weeks apart, 10 weeks apart, 3 months apart, or 6 months apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the initiation of the induction phase. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some

embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. In some embodiments, the maintenance phase comprises 7 treatment cycles. In some embodiments, the subject is administered the chimeric poliovirus in a maintenance phase only. In some embodiments, the maintenance phase is administered following the cessation of an induction phase. In some embodiments, the maintenance phase is administered following an objective response rate (ORR) exhibited by the patient following the cessation of the induction phase. In some embodiments, the maintenance phase is administered following a complete response (CR) exhibited by the patient following the cessation of the induction phase. In some embodiments, the method is administered until disease progression or death. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In one aspect, an effective amount of a chimeric poliovirus is administered to a subject with bladder cancer during an induction phase as described above, and, following the cessation of the induction phase, an effective amount of a chimeric poliovirus is further administered to the subject in a maintenance phase as described above. In some embodiments, the maintenance phase is administered until disease progression or death. In some embodiments, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 3 weeks, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 2 weeks, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 1 week, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least

10, or 11 or more treatment cycles. In some embodiments, the maintenance phase is administered until disease progression or death. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In some embodiments, each treatment cycle of the maintenance phase is administered at least 4 weeks apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase. In some embodiments, each treatment cycle of the maintenance phase is administered at least 4 weeks apart, at least 6 weeks apart, at least 8 weeks apart, at least 10 weeks apart, at least 12 weeks apart, or greater than 12 weeks apart. In some embodiments, each treatment cycle of the maintenance phase is administered at least 1 month apart, at least 2 months apart, at least 3 months apart, at least 4 months apart, at least 5 months apart, at least 6 months apart, or a combination thereof. In some embodiments, the subject is administered a maintenance phase alone. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

As provided herein, the chimeric poliovirus for administration by intravesical instillation in the methods described herein can be administered, for example, as a pharmaceutical composition that includes an effective amount of the chimeric poliovirus, for example lerapolturev, for a subject, typically a human, in need of such treatment in a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical composition comprises at least one chimeric poliovirus administered at a high dose by intravesical instillation, for example at a total dose of between about $2.0x10^8$ $TCID_{50}$ and about $5.0x10^{10}$ $TCID_{50}$, for example up to about $2.0x10^8$ $TCID_{50}$, up to about $2.0x10^9$ $TCID_{50}$, up to about $5.0x10^{10}$ $TCID_{50}$. In some embodiments, the total dose administered at each intravesical instillation is between about $2.0x10^8$ $TCID_{50}$ and about $1.0x10^{10}$ $TCID_{50}$. In some embodiments, the total dose administered at each intravesical instillation is about $8.0x10^8$ to about $1.0x10^{10}$ $TCID_{50}$. In some embodiments, the methods described herein can be used to treat one or more solid bladder tumor(s) comprising administering a chimeric poliovirus at a dose of between about $8.0x10^8$ to about $1.0x10^9$, about $1.0x10^9$ to about $3.0x10^9$, about $3.0x10^9$ to about $5.0x10^9$ $TCID_{50}$, about $5.0x10^9$ to about $7.0x10^9$ $TCID_{50}$, or about $7.0x10^9$ to about $1.0x10^{10}$

$TCID_{50}$. In some embodiments, the methods described herein can be used to treat a solid tumor comprising administering a chimeric poliovirus construct at a dose of about $2.0x10^9$ $TCID_{50}$ per administration. In some embodiments, the pharmaceutically acceptable carrier comprises between about 20 nM and about 80 nM sodium phosphate in between about 0.5% and about 1.5% sodium chloride, with a pH of between about pH 6.8 to about pH 7.8, with between about 0.1% and about 0.5% human serum albumin (HSA) in phosphate buffered saline (PBS). In some embodiments, the pharmaceutically acceptable carrier comprises about 50 mM sodium phosphate in about 0.9% sodium chloride, about pH 7.4 with about 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS).

In some embodiments, the pharmaceutical composition is retained in the bladder of the patient for between about 30 minutes to about 2 hours. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for about 30 minutes. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for about 45 minutes. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for about 1 hour. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for about 90 minutes. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for about 2 hours. In some embodiments, the instilled suspension comprising a chimeric poliovirus has sufficient contact with the whole mucosal surface of the bladder. In some embodiments, the patient is mobilized about every 15 minutes. In some embodiments, the patient is lying down and rotates between prone, supine, left lateral, and right lateral positions about every 15 minutes. In some embodiments, the pharmaceutical composition is retained in bladder of the patient for more than about 2 hours.

In some embodiments, the chimeric poliovirus is administered within 7 days of a transurethral resection of a bladder tumor (TURBT). In some embodiments, the chimeric poliovirus is administered within 7 days prior to a TURBT. In some embodiments, the chimeric poliovirus is administered within 7 days following a TURBT. In some embodiments, the chimeric poliovirus is administered within about 24 hours of a TURBT. In some embodiments, the chimeric poliovirus is administered within 24 hours prior to a TURBT. In some embodiments, the chimeric poliovirus is administered within about 24 hours following a TURBT. In some embodiments, the chimeric poliovirus is lerapolturev. In some embodiments, the bladder cancer is non-muscle

invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In an alternative aspect, provided herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises a single administration of an effective amount of a chimeric poliovirus intravesical instillation, wherein the chimeric poliovirus is administered within 24 hours of transurethral resection of the bladder tumor (TURBT). In some embodiments, the chimeric poliovirus is administered within 24 hours prior to a TURBT. In some embodiments, the chimeric poliovirus is administered within about 24 hours following a TURBT. In some embodiments, the chimeric poliovirus is lerapolturev. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

As provided herein, the chimeric poliovirus can be lerapolturev, also known as PVSRIPO. The nucleic acid sequence of lerapolturev is provided in SEQ ID NO:1. In some embodiments, the chimeric poliovirus administered according to the methods provided herein comprises a nucleic acid sequence of SEQ ID NO:1, or a nucleic acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

In one aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising a 6-week treatment cycle comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week. In some embodiments, the induction cycle is repeated two or more times. In some embodiments the treatment cycle is repeated two times. In some embodiments, the treatment cycle is repeated at least 2 times, at least 3 times, at least 4 times, or up to 5 times. In some embodiments, the method is administered until disease progression or death. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In one aspect, the induction phase comprises 4-week treatment cycles comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each cycle. In some embodiments, the 4-week treatment cycle is repeated 2, 3, 4, or more than 4 times. In some embodiments, the method is administered until disease

progression or death. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In another aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising two 6-week treatment cycles, wherein each 6-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In another aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase and a maintenance phase, the induction phase comprising a 6-week treatment cycle comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week and the maintenance phase comprising 3-week treatment cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, each 3-week treatment cycle comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week, and, wherein the maintenance phase is administered following the cessation of the induction phase.

In still another aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase and a maintenance phase, the induction phase comprising 4-week induction cycles comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each cycle, wherein the cycle is repeated three times, and the maintenance phase comprising 3-week treatment cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, each 3-week treatment cycle comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week, and, wherein the maintenance phase is administered following the cessation of the induction phase.

In some embodiments provided herein, the bladder cancer for treatment is selected from a non-muscle invasive bladder cancer (NMIBC) or a muscle invasive bladder cancer (MIBC). In some embodiments, the bladder cancer is a non-muscle invasive bladder cancer (NMIBC). In

some embodiments, the NMIBC is selected from Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient is ineligible for cystectomy, Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient who has elected not to undergo cystectomy, Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient is ineligible for cystectomy, or Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient who has elected not to undergo cystectomy In some embodiments, the MIBC is selected from resectable cisplatin-ineligible/refusal MIBC, or locally advanced or metastatic bladder cancer that has not progressed with first-line platinum-containing chemotherapy. In some embodiments, the subject has refused or is ineligible for cisplatin-based therapy with glomerular filtration rate (GFR) < 60 mL/min calculated per institutional standard. In some embodiments, the subject has refused or is ineligible for cisplatin-based therapy with Common Terminology Criteria for Adverse Events (CTCAE) v 5.0 Grade ≥ 2 hearing loss. In some embodiments, the subject has refused or is ineligible for cisplatin-based therapy with CTCAE v 5.0 Grade ≥ 2 peripheral neuropathy. In some embodiments, the subject previously received cancer therapy. In some embodiments, the subject previously received cancer therapy. In some embodiments, the previously received cancer therapy is selected from exposure to an intravesical agent, a radiation therapy, a chemotherapeutic agent, an immune checkpoint inhibitor (ICI), or a combination thereof.

In some embodiments, prior to administration of the first dose of the chimeric poliovirus, a subject is first administered a boost immunization of a poliovirus vaccine, for example, at least 1 week, but less than 6 weeks, prior to day 1 of the first induction phase cycle. Suitable poliovirus vaccines for administration prior to the initiation of the induction phase include trivalent IPOL® (Sanofi-Pasteur SA).

In some embodiments, the method further comprises administering an adjuvant with the chimeric poliovirus. In some embodiments, the adjuvant comprises a detergent. In some embodiments, the detergent comprises N-dodecyl-β-D-maltoside (DDM). In some embodiments, the detergent comprises Tween-80. In some embodiments, the detergent comprises SIM3. In some embodiments, the administration of detergent increases cellular viral intake. In some embodiments, the detergent is administered one or more times as a pre-wash. In some

embodiments, the detergent is administered with a chimeric poliovirus by intravesical administration. In some embodiments, the pharmaceutical composition described herein further comprises detergent. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration between at about 0.1% and at about 1.0%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 0.1%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 0.5%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 1.0%. In some embodiments, the detergent is administered at a concentration of from about 2% to about 6%. In some embodiments, the detergent is administered at a concentration of about 5%.

In some embodiments, the chimeric poliovirus is administered via intravesical instillation following a pre-wash sequence. The pre-wash sequence may increase or enhance the transduction of the chimeric poliovirus. In some embodiments, the pre-wash results in the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the epithelium. In some embodiments, the pre-wash comprises a surfactant that disrupts the GAG layer, thereby providing access to the underlying epithelium of the bladder. In some embodiments, the surfactant is a mild polar surfactant. In some embodiments, the surfactant may be, but is not limited to, Tween-80, sodium dodecyl sulfate, or cetylpyridinium chloride. In some embodiments, the pre-wash comprises a calcium ion chelator that disrupts the GAG layer, thereby providing access to the underlying epithelium of the bladder. In some embodiments, the calcium ion chelator may be, for example, polycarbophil.

In some embodiments, the pre-wash sequence comprises one or more n-dodecyl-B-D-maltoside (DDM) washes and one or more saline washes. In some embodiments, the DDM pre-wash comprises between about 0.5% and about 10% DDM. In some embodiments, the pre-wash comprises between about 2% and about 6% of DDM. In some embodiments, the pre-wash comprises about 5% DDM. In some embodiments, a saline wash is administered prior to the DDM wash. In some embodiments, a saline wash is administered after the DDM wash. In some embodiments, a saline wash is administered prior to the DDM wash and then again after the saline wash. In some embodiments, the saline wash is administered and retained within the bladder for from about 2 minutes to about 10 minutes. In some embodiments, the saline wash is administered

and retained within the bladder for about 5 minutes. In some embodiments, the DDM wash is administered and retained within the bladder from about 2 minutes to about 25 minutes. In some embodiments, the DDM wash is administered and retained within the bladder for from about 10 minutes to about 20 minutes. In some embodiments, the DDM wash is administered and retained in the bladder for about 15 minutes +/- about 5 minutes. In some embodiments, the DDM wash is administered and retained in the bladder for about 5 minutes.

In some embodiments, prior to the administration of the chimeric poliovirus via intravesical instillation, the patient is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, iii) a third wash comprising about 100 ml saline. In some embodiments, prior to the administration of the chimeric poliovirus via intravesical instillation, the patient is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, iii) a third wash comprising about 100 ml saline, wherein each saline wash is administered and retained in the bladder for about 5 minutes and the DDM wash is administered and retained in the bladder for about 15 minutes +/- about 5 minutes.

In some embodiments, the chimeric poliovirus is further administered in combination with an ICI. The improved treatment methods described herein block tumor infiltrating immune effector cell immune checkpoint expression downregulation to prevent tumor immune escape, resulting in an extended or prolonged efficacy of an anti-cancer regimen. The administration of an effective amount of a chimeric poliovirus and an effective amount of an ICI are capable of synergizing to reverse and/or significantly delay the growth of tumors and/or the development of ICI therapy resistance. Suitable ICIs for use in the methods described herein include, but are not limited to, a programmed cell death-1 (PD-1) inhibitor, a programmed cell death-ligand 1 (PD-L1) inhibitor, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, a lymphocyte-activation gene 3 (LAG-3) inhibitor, a T-cell immunoglobulin mucin-3 (TIM-3) inhibitor, a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor, a programmed cell death-ligand 2 (PD-L2) inhibitor, a V-domain Ig suppressor of T-cell activation (VISTA) inhibitor, a B7-H3/CD276 inhibitor, an indoleamine 2,3-dioxygenase (IDO) inhibitor, a killer immunoglobulin-like receptor (KIR) inhibitor, a carcinoembryonic antigen cell adhesion molecule (CEACAM) inhibitor against molecules such as CEACAM-1, CEACAM-3, and CEACAM-5, a sialic acid-

binding immunoglobulin-like lectin 15 (Siglec-15) inhibitor, a CD47 inhibitor, a CD39 inhibitor, or a B and T lymphocyte attenuator (BTLA) protein inhibitor, or a combination thereof. Furthermore, the improved treatment methods described herein provide enhanced therapeutic efficacy through the regulation of T cells, including activation of cytotoxic CD8+ T-cell function and maturation into memory CD8+ T-cells. The improved treatment methods described herein that combine administration of a chimeric poliovirus construct at certain doses and interval frequencies in combination with administering an effective amount of an ICI at certain doses and interval frequencies provide anti-tumor potency and measurable reductions in tumor progression. In some embodiments, the ICI is administered at the standard recommended dose and schedule as described on its FDA approved label.

In some embodiments, the method further comprises administering an anti-cancer therapy selected from an intravesical agent, a radiation therapy, a chemotherapeutic agent, or a combination thereof.

In some embodiments, the chimeric poliovirus can be administered intratumorally between induction or maintenance phase treatment cycles.

In some embodiments, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising a 6-week cycle comprising administering intratumorally to the patient an effective amount of a chimeric poliovirus on the first day of each week.

In some embodiments, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising two 6-week cycles, each 6-week cycle comprising administering intratumorally to the patient an effective amount of a chimeric poliovirus on the first day of each week.

In some embodiments, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase and a maintenance phase, the induction phase comprising a 6-week cycle comprising administering intratumorally to the patient an effective amount of a chimeric poliovirus on the first day of each week the maintenance phase comprising 3-week cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, each 3-week cycle comprising administering intratumorally to the

patient an effective amount of a chimeric poliovirus on the first day of each week, and, wherein the maintenance phase is administered following the cessation of the induction phase.

In some embodiments, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase and a maintenance phase, the induction phase comprising a 6-week cycle comprising administering intratumorally to the subject an effective amount of a chimeric poliovirus on the first day of each week. the maintenance phase comprising 1-week cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, each 1-week cycle comprising administering intratumorally to the subject an effective amount of a chimeric poliovirus on the first day of each week, and, wherein the maintenance phase is administered following the cessation of the induction phase.

In some embodiments of the above intratumoral injection protocols, the chimeric poliovirus is administered within 7 days of a TURBT. In some embodiments, the induction phase is repeated one or more times. In some embodiments, the maintenance phase follows immediately after the induction phase. In some embodiments, the methods described herein can be used to treat a solid bladder tumor comprising administering the chimeric poliovirus to between 1 and 10 lesions. In some embodiments, the chimeric poliovirus is administered to at least 1 lesion, at least 2 lesions, at least 3 lesions, at least 4 lesions, at least 5 lesions, at least 6 lesions, at least 7 lesions, at least 8 lesions, at least 9 lesions, or up to 10 lesions per administration.

The administration of a treatment protocol described herein may provide enhanced anti-tumor efficacy in patients. In some embodiments, the administration of a treatment protocol described herein provides improved progression free survival (PFS) and/or overall survival (OS) compared to a patient receiving cystectomy or TURBT alone. In some embodiments, an improvement in PFS is observed. In some embodiments, an improvement in OS is observed.

In some embodiments, the methods described herein reduce recurrence rate of bladder tumor formation. In some embodiments, the methods described herein decrease the need of bladder tumor removal surgeries selected from transurethral resection of the bladder tumor (TURBT) or cystectomy. In some embodiments, the methods described herein decrease the frequency of TURBT. In some embodiments, the methods described herein decrease the frequency of cystectomy.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A – E shows that chemical conditions do not significantly alter lerapolturev infectivity and immunomodulation. The U87 cell death assay was conducted at multiplicity of infection values (MOI) of 20, 6, and 2.2 tested in triplicate.

FIG. 1A The effect of a 2-hour incubation of cells with Tween-80 on lerapolturev infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different MOI groups. Concentration of Tween-80 ranged from 0.1% - 1.0%.

FIG. 1B The effect of a 2-hour incubation of cells with n-dodecyl-B-D-maltoside (DDM) on lerapolturev infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different MOI groups. Concentration of DDM ranged from 0.1% - 1.0%.

FIG. 1C The effect of a 2-hour incubation of cells with varying media pH conditions on lerapolturev infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different MOI groups. pH values were 3, 7, and 10.

FIG. 1D The effect of a 2-hour incubation of cells with urine on lerapolturev infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different MOI groups. Concentration of urine ranged from 11% - 100%.

FIG. 1E The effect of a 2-hour incubation of cells with saliva on lerapolturev infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different MOI groups. Concentration of saliva ranged from 11% - 100%.

FIG.2A – E shows that chemical conditions do not significantly dampen levels of interferon beta (IFN- β) secreted from cells infected with lerapolturev as measured by ELISA.

FIG. 2A is a bar plot showing the effect of varying concentration of detergent Tween-80 on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. Different multiplicity of infection (MOI) values (2, 6.6, 20) and experimental groups (GM-PC, Tween-80 0.1%, 0.5%, 1.0%) are also illustrated above the graph for ease of viewing. GM, Growth Media.

FIG. 2B is a bar plot showing the effect of varying concentration of detergent n-dodecyl-B-D-maltoside (DDM) on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. Different multiplicity of infection (MOI) values (2, 6.6, 20) and experimental groups (GM-PC, DDM 0.1%, 0.5%, 1.0%) are also illustrated above the graph for ease of viewing.

FIG. 2C is a bar plot showing the effect of varying concentration of Urine on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. Different multiplicity of infection (MOI) values (2, 6.6, 20) and experimental groups (GM-PC, Urine 11%, 37%, 56%, 100%) are also illustrated above the graph for ease of viewing.

FIG. 2D is a bar plot showing the effect of varying concentration of urine on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. Different multiplicity of infection (MOI) values (2, 6.6, 20) and experimental groups (GM-PC, Urine 11%, 37%, 56%, 100%) are also illustrated above the graph for ease of viewing.

FIG. 2E is a bar plot showing the effect of varying concentration of Saliva on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. Different multiplicity of infection (MOI) values (2, 6.6, 20) and experimental groups (GM-PC, Saliva 11%, 37%, 56%, 100%) are also illustrated above the graph for ease of viewing.

FIG. 3A – C shows that a minimum of 30 minutes of lerapolturev contact of cells is feasible for successful lerapolturev infection.

FIG. 3A is a diagram illustrating the experimental design showing the timeline of lerapolturev incubation time lengths. The x-axis represents the time length of the experiment, totaling 44 hours. The y-axis represents different experimental groups with different time lengths of lerapolturev cell contact, ranging from no time (neg control) to 44 hours (pos control). Black arrows represent the lengths of time lerapolturev is contacting cells, while white arrows represent the lengths of time fresh media (absent lerapolturev) is contacting cells in each experimental condition.

FIG. 3B is a bar plot showing the effect of varying the length of lerapolturev incubation on lerapolturev cell infectivity was analyzed using the U87 Potency Assay. The y-axis represents the Absorbance value of each experimental group, while the x-axis represents the experimental conditions in different multiplicity of infection groups.

FIG. 3C is a bar plot showing the effect of varying lengths of lerapolturev contact time on the levels of IFN-β secreted by infected cells as measured by ELISA. The y-axis represents the amount of secreted IFN-β in picograms/milliliter (pg/mL), while the x-axis represents the different experimental groups. MOI, multiplicity of infection.

FIG. 4 is a flow chart diagram showing the dose confirmation scheme to identify the exemplary dose of lerapolturev to be administered in a proposed clinical trial. The exemplary dose of lerapolturev to be administered by intravesical instillation will be determined using a 3+3 dose escalation approach. Three patients initially treated with a dose of $2.0x10^9$ $TCID_{50}$ and the frequency of dose limiting toxicity events will be assessed. The decision to escalate or de-escalate the lerapolturev dose will be determined by the number of DLTs observed during the initial 14 days following administration of lerapolturev as advised by the Data Safety Monitoring Committee (DSMC). If no DLTs are observed the dose will be considered to not have exceeded the maximally tolerated dose (MTD) and a new cohort of patients (n=3) would then be administered a total dose of $1.0x10^{10}$ $TCID_{50}$ by intravesical instillation. If, however, 1 out of 3 patients initially enrolled experience a DLT, the cohort would be expanded by an additional 3 patients. If no additional patients experience a DLT in the second cohort, a new cohort of patients (n=3) would then be administered a total dose of $1.0x10^{10}$ $TCID_{50}$ by intravesical instillation. If a 2nd patient from the

originally enrolled cohort experience a DLT, or among the expanded 6 patient enrollment, the dose will be de-escalated to $2.0x10^8$ $TCID_{50}$.

FIG. 5A – D show exemplary intravesical instillation administration schedules of a chimeric poliovirus as provided herein.

FIG. 5A is an exemplary chimeric poliovirus administration schedule comprising an induction (I) phase lasting one six-week Induction cycle (IC1). A chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of each week of IC1.

FIG. 5B is an exemplary chimeric poliovirus administration schedule comprising an induction (I) phase lasting two separate six-week Induction cycles (IC1 + IC2). A chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of each week of IC1 and IC2.

FIG. 5C is an exemplary chimeric poliovirus administration schedule comprising an induction (I) phase and a maintenance (M) phase. A chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of each week of the six-week IC1. Following the cessation of the I Phase, the M phase begins, in which a chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of each of the first three weeks of months 3, 6, 12, 18, 24, 30, and 36.

Fig. 5D is an exemplary chimeric poliovirus administration schedule comprising an induction (I) phase and an alternate maintenance (M) phase. A chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of each week of the six-week IC1. Following the cessation of the I Phase, the M phase begins, in which a chimeric poliovirus (e.g., lerapolturev) is to be administered on the first day of the first week of months 3, 6, 12, 18, 24, 30, and 36.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods for treating a human patient having a cancer or which are unresponsive to previous therapy. For example, multiple administrations of a chimeric poliovirus can be administered by intravesical instillation or at another suitable delivery area to a patient having a bladder cancer and/or one or more disease or disorders associated with bladder tumors or which are unresponsive to previous therapy.

Terminology

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Each of the references cited herein are incorporated by reference in its entirety.

The terms "a" and "an" do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced item. The term "or" means "and/or". Recitation of ranges of values are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. The endpoints of all ranges are included within the range and independently combinable. All methods described herein can be performed in a suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of examples, or exemplary language (e.g., "such as"), is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. Unless defined otherwise, technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs.

As used herein, the term "about" means ± 10%.

The "patient" or "subject" or "participant" treated is typically a human patient, although it is to be understood the methods described herein are effective with respect to other animals, such as mammals. More particularly, the term patient can include animals used in assays such as those used in preclinical testing including but not limited to mice, rats, monkeys, dogs, pigs, and rabbits; as well as domesticated swine (pigs and hogs), ruminants, equine, poultry, felines, bovines, murines, canines, and the like.

An "effective amount" as used herein, means an amount which provides a therapeutic or prophylactic benefit.

To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease, disorder, or side-effect experienced by a patient (i.e., palliative treatment) or to decrease a cause or effect of the disease, disorder (i.e., disease-modifying treatment), or side effect experienced by a patient as a result of the administration of a therapeutic agent.

As used herein, the term "detergent" means a surfactant or mixture of surfactants with amphiphilic structures, wherein each molecule has a hydrophilic (polar) head and a long hydrophobic (non-polar) tail

As used herein, the term "response evaluation criteria in solid tumors version 1.1 (RECIST 1.1)" refers to a revised guideline that describes a standard approach to solid tumor measurements and definitions for objective change in tumor size for use in trials in which an immunotherapy is used (Eisenhauer et al. Eur J Cancer.45:228-47(2009)).

As used herein, the term "iRECIST" refers to a consensus guideline that describes a standard approach to solid tumor measurements and definitions for objective change in tumor size for use in trials in which an immunotherapy is used (Seymour et al. Lancet Oncol. 18(3):30074-8(2019)).

As used herein, the term "complete response (CR)" refers to the disappearance of all target lesions per RECIST 1.1.

As used herein, the term "partial response (PR)" refers to greater than or equal to 30% decrease in the sum of the longest diameters of target lesions compared with baseline per RECIST 1.1.

As used herein, the term "progressive disease (PD)" refers to a 5-mm absolute increase of the sum of the longest diameters of the target lesions in addition to greater than or equal to 20% increase in the sum of the longest diameter of target lesions compared with the smallest-sum longest diameter recorded or the appearance of one or more new lesions per RECIST 1.1.

As used herein, the term "stable disease (SD)" refers to neither PR or PD occurring when evaluating target lesions per RECIST 1.1.

As used herein, the term "overall survival (OS)" refers to the time from treatment group assignment until death from any cause.

As used herein, the term "duration of response (DOR)" refers to time from confirmed objective response (CR or PR per RECIST 1.1) until unequivocal disease progression or death, whichever occurs first.

As used herein, the term "disease control rate (DCR)" refers to the proportion of patients achieving confirmed CR, confirmed PR, or SD per RECIST 1.1 as best response.

As used herein, the term "disease control rate-6months (DCR-6mo)" refers to the proportion of patients achieving confirmed CR (for any duration), confirmed PR (for any duration), or SD (greater than or equal to 6 months) per RECIST 1.1 as best response.

As used herein, the term "durable response rate" refers to the proportion of patients with confirmed CR or PR (per RECIST 1.1) last at least 6 months.

As used herein, the term "progression-free survival (PFS)" refers to the time (i.e., number of months) from treatment group assignment until date of documented radiologic disease progression per RECIST 1.1 or death due to any cause, whichever comes first.

The terms "percent identical," "percent homologous," or "percent similarity", and the like, when used in the context of nucleic acid sequences refers to the residues in the two sequences being compared which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the sequence, or, or alternatively a fragment of at least about 50 to 2500 nucleotides. Similarly, the terms "percent identical," "percent homologous," or "percent similarity", may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length and may be up to about 7500 amino acids. Examples of suitable fragments are described herein. Generally, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences. "Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. Alignments can be performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Examples of such programs include, "Clustal Omega", "Clustal W", "CAP Sequence Assembly", "MAP", and "MEME", which are accessible through Web Servers on the

internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™, a program in GCG Version 6.1. Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Multiple sequence alignment programs are also available for amino acid sequences, e.g., the "Clustal Omega", "Clustal X", "MAP", "PIMA", "MSA", "BLOCKMAKER", "MEME", and "Match-Box" programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, e.g., J. D. Thomson et al, Nucl. Acids. Res., "A comprehensive comparison of multiple sequence alignments", 27(13):2682-2690 (1999).

Throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and should not be construed as a limitation on the scope of the invention. The description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Chimeric Poliovirus

Methods for treating bladder cancer are provided herein comprising administering to a subject, generally a human, an effective amount of a chimeric poliovirus. In one embodiment, the chimeric poliovirus is a modified serotype 1 live attenuated (Sabin™) PV vaccine (PV1S) with the cognate internal ribosome entry site (IRES) replaced with that of human rhinovirus type 2

(HRV2), for example lerapolturev, also known as PVSRIPO. The nucleic acid sequence of lerapolturev is provided in Table 2 (SEQ ID NO:1).

Lerapolturev is a recombinant rhinovirus/poliovirus chimera developed to treat patients with solid tumor cancers. The foreign IRES of PVSRIPO causes neuronal incompetence: a failure to recruit host ribosomes, translate viral genomes, and propagate in neurons, each of which contribute to the ablation of neurovirulence and absence of polio-related neurologic injury (Dobrikova et al. J Virol. 86(5):2750-9(2012)). Lerapolturev exhibits selective infectivity and cytotoxicity towards CD155-expressing cells, which includes malignant cells of virtually all solid tumors (Luo et al. Front Oncol. 11:660273(2021); Takai et al. Nat Rev Mol Cell Biol. 9:603-15(2008); Chandramohan et al. Arch Pathol Lab Med. 141(12):1697-1704(2017); Liu et al. 11(15):5463-82(2019); Masson et al. Gut. 49:236-40(2001); Bevelacqua et al. Oncotarget. 3(8):882-92(2012); Carlsten et al. J Immunol. 183(8):4921-30(2009); Nishiwada et al. Anticancer Research. 35:2287-98(2015); Sun et al. International Immunopharmacology. 80:106198(2020); Zhang et al. Urol Oncol. 38(2):41(2020)). Among these CD155-expressing solid tumors are cancers of the bladder, including both muscle invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC).

Table 2. Lerapolturev/PVSRIPO Sequence

SEQ ID NO:	Sequence
1 - lerapolturev/ PVSRIPO	TTAAAACAGCTCTGGGGTTGTACCCACCCCAGAGGCCCACGTGGCGGCTAGTACTCC GGTATTGCGGTACCCTTGTACGCCTGTTTTATACTCCCTTCCCGTAACTTAGGAATTC AACTTAGAAGTTTTTCACAAAGACCAATAGCCGGTAATCAGCCAGATTACTGAAGG TCAAGCACTTCTGTTTCCCCGGTCAATGTTGATATGCTCCAACAGGGCAAAAACAAC TGCGATCGTTAACCGCAAAGCGCCTACGCAAAGCTTAGTAGCATCTTTGAAATCGTT TGGCTGGTCGATCCGCCATTTCCCCTGGTAGACCTGGCAGATGAGGCTAGAAATACC CCACTGGCGACAGTGTTCTAGCCTGCGTGGCTGCCTGCACACCCTATGGGTGTGAAG CCAAACAATGGACAAGGTGTGAAGAGCCCCGTGTGCTCGCTTTGAGTCCTCCGGCC CCTGAATGTGGCTAACCTTAACCCTGCAGCTAGAGCACGTAACCCAATGTGTATCTA GTCGTAATGAGCAATTGCGGGATGGGACCAACTACTTTGGGTGTCCGTGTTTCACTT TCTCCTTTATATTTGCTTATGGTGACAATATATACAATATATATATTGGCACCATGGG AGCTCAGGTTTCATCACAGAAAGTGGGCGCACATGAAAACTCAAATAGAGCGTATG GTGGTTCTACCATTAATTACACCACCATTAATTATTATAGAGATTCAGCTAGTAACG CGGCTTCGAAACAGGACTTCTCTCAAGACCCTTCCAAGTTCACCGAGCCCATCAAGG ATGTCCTGATAAAAACATCCCCAATGCTAAACTCGCCAAACATAGAGGCTTGCGGG TATAGCGATAGAGTACTGCAATTAACACTGGGAAACTCCACTATAACCACACAGGA GGCGGCTAATTCAGTAGTCGCTTATGGGCGTTGGCCTGAATATCTGAGGGACAGCG AAGCCAATCCAGTGGACCAGCCGACAGAACCAGACGTCGCTGCATGCAGGTTTTAT ACGCTAGACACCGTGTCTTGGACGAAAGAGTCGCGAGGGTGGTGGTGGAAGTTGCC TGATGCACTGCGGGACATGGGACTCTTTGGCCAAAATATGTACTACCACTACCTAGG TAGGTCCGGGTACACCGTGCATGTACAGTGTAACGCCTCCAAATTCCACCAGGGGG

	CACTAGGGGTATTCGCCGTACCAGAGATGTGTCTGGCCGGGGATAGCAACACCACT ACCATGCACACCAGCTATCAAAATGCCAATCCTGGCGAGAAAGGAGGCACTTTCAC GGGTACGTTCACTCCTGACGACAACCAGACATCACCTGCCCGTAGGTTCTGCCCGGT GGATTACCTCTTTGGAAATGGCACGTTATTGGGGAATGCCTTTGTGTTCCCGCACCA GATAATAAACCTACGGACCAACAACTGTGCTACACTGGTACTCCCTTACGTGAACTC CCTCTCGATAGATAGTATGGTAAAGCACAATAATTGGGGAATTGCAATATTACCATT GGCCCCATTAAATTTTGCTAGTGAGTCCTCCCCAGAGATTCCAATCACCTTGACCAT AGCCCCTATGTGCTGTGAGTTCAATGGATTAAGAAACATTACCCTGCCACGCTTACA GGGCCTGCCGGTCATGAACACCCCTGGTAGCAATCAATATCTTACTGCAGACAACTT CCAGTCACCGTGTGCGCTGCCTGAATTTGATGTGACCCCACCTATTGACATACCCGG TGAAGTTAAGAACATGATGGAATTGGCAGAAATCGACACCATGATTCCCTTTGACTT AAGTGCAAAAAAAAAGAACACCATGGAAATGTATAGGGTTCGGTTAAGTGACAAAC CACATACAGACGATCCCATACTCTGCCTGTCACTCTCTCCAGCTTCAGATCCTAGGT TGTCACATACTATGCTTGGAGAAATCCTAAATTACTACACACACTGGGCAGGATCCC TGAAGTTCACGTTTCTGTTCTGTGGATCCATGATGGCAACTGGCAAACTGTTGGTGT CATACGCGCCTCCTGGAGCCGACCCACCAAAGAAGCGTAAGGAGGCGATGTTGGGA ACACATGTGATCTGGGACATAGGACTGCAGTCCTCATGTACTATGGTAGTGCCATGG ATTAGCAACACCACGTATCGGCAAACCATAGATGATAGTTTCACCGAAGGCGGATA CATCAGCGTCTTCTACCAAACCAGAATAGTCGTCCCTCTTTCGACACCCAGAGAGAT GGACATCCTTGGTTTTGTGTCAGCGTGTAATGACTTCAGCGTGCGCTTGATGCGAGA TACCACACATATAGAGCAAAAAGCGCTAGCACAGGGGTTAGGTCAGATGCTTGAAA GCATGATTGACAACACAGTCCGTGAAACGGTGGGGGCGGCAACGTCTAGAGACGCT CTCCCAAACACTGAAGCCAGTGGACCAGCACACTCCAAGGAAATTCCGGCACTCAC CGCAGTGGAAACTGGGGCCACAAATCCACTAGTCCCTTCTGATACAGTGCAAACCA GACATGTTGTACAACATAGGTCAAGGTCAGAGTCTAGCATAGAGTCTTTCTTCGCGC GGGGTGCATGCGTGGCCATTATAACCGTGGATAACTCAGCTTCCACCAAGAATAAG GATAAGCTATTTACAGTGTGGAAGATCACTTATAAAGATACTGTCCAGTTACGGAGG AAATTGGAGTTCTTCACCTATTCTAGATTTGATATGGAATTTACCTTTGTGGTTACTG CAAATTTCACTGAGACTAACAATGGGCATGCCTTAAATCAAGTGTACCAAATTATGT ACGTACCACCAGGCGCTCCAGTGCCCGAGAAATGGGACGACTACACATGGCAAACC TCATCAAATCCATCAATCTTTTACACCTACGGAACAGCTCCAGCCCGGATCTCGGTA CCGTATGTTGGTATTTCGAACGCCTATTCACACTTTTACGACGGTTTTTCCAAAGTAC CACTGAAGGACCAGTCGGCAGCACTAGGTGACTCCCTCTATGGTGCAGCATCTCTAA ATGACTTCGGTATTTTGGCTGTTAGAGTAGTCAATGATCACAACCCGACCAAGGTCA CCTCCAAAATCAGAGTGTATCTAAAACCCAAACACATCAGAGTCTGGTGCCCGCGT CCACCGAGGGCAGTGGCGTACTACGGCCCTGGAGTGGATTACAAGGATGGTACGCT TACACCCCTCTCCACCAAGGATCTGACCACATATGGATTCGGACACCAAAACAAAG CGGTGTACACTGCAGGTTACAAAATTTGCAACTACCATTTGGCCACTCAGGAAGATT TGCAAAACGCAGTGAACGTCATGTGGAATAGAGACCTCTTAGTCACAGAATCAAGA GCCCAGGGCACCGATTCAATCGCAAGGTGCAATTGCAACGCAGGGGTGTACTACTG CGAGTCTAGAAGGAAATACTACCCAGTATCCTTCGTTGGCCCAACGTTCCAGTACAT GGAGGCTAATAACTATTACCCAGCTAGGTACCAGTCCCATATGCTCATTGGCCATGG ATTCGCATCTCCAGGGGATTGTGGTGGCATACTCAGATGTCACCACGGGGTGATAGG GATCATTACTGCTGGTGGAGAAGGGTTGGTTGCATTTACAGACATTAGAGACTTGTA TGCCTACGAAGAAGAAGCCATGGAACAAGGCATCACCAATTACATAGAGTCACTTG GGGCCGCATTTGGAAGTGGATTTACTCAGCAGATTGGAGACAAAATAACAGAGTTG ACTAATATGGTGACCAGTACCATCACTGAAAAGCTACTTAAGAACTTGATCAAGATC ATATCCTCACTAGTTATTATAACTAGGAATTATGAAGACACCACAACAGTGCTCGCT ACCCTGGCCCTTCTTGGGTGTGATGCTTCACCATGGCAGTGGCTTAGAAAGAAAGCA TGCGATGTTCTGGAGATACCTTATGTCACCAAGCAAGGTGACAGTTGGTTGAAGAA GTTTACTGAAGCATGCAACGCAGCTAAGGGACTGGAGTGGGTGTCAAACAAAATCT CAAAATTCATTGATTGGCTCAAGGAGAAAATTATCCCACAAGCTAGAGATAAGTTG GAATTTGTAACAAAACTTAGACAACTAGAAATGCTGGAAAACCAAATCTCAACTAT ACACCAATCATGCCCTAGTCAGGAACACCAGGAAATTCTATTCAATAATGTCAGATG GTTATCCATCCAGTCTAAGAGGTTTGCCCCTCTTTACGCAGTGGAAGCCAAAAGAAT

	ACAGAAACTAGAGCATACCATTAACAACTACATACAGTTCAAGAGCAAACACCGTA TTGAACCAGTATGTTTGCTAGTACATGGCAGCCCCGGAACAGGTAAATCTGTAGCAA CCAACCTGATTGCTAGAGCCATAGCTGAAAGAGAAAACACGTCCACGTACTCGCTA CCCCCGGATCCATCACACTTCGACGGATACAAACAACAGGGAGTGGTGATTATGGA CGACCTGAATCAAAACCCAGATGGTGCGGACATGAAGCTGTTCTGTCAGATGGTAT CAACAGTGGAGTTTATACCACCCATGGCATCCCTGGAGGAGAAAGGAATCCTGTTT ACTTCAAATTACGTTCTAGCATCCACGAACTCAAGCAGAATTTCCCCCCCCCACTGTG GCACACAGTGATGCATTAGCCAGGCGCTTTGCGTTCGACATGGACATTCAGGTCATG AATGAGTATTCTAGAGATGGGAAATTGAACATGGCCATGGCTACTGAAATGTGTAA GAACTGTCACCAACCAGCAAACTTTAAGAGATGCTGTCCTTTAGTGTGTGGTAAGGC AATTCAATTAATGGATAAATCTTCCAGAGTTAGATACAGTATTGACCAGATCACTAC AATGATTATCAATGAGAGAAACAGAAGATCCAACATTGGCAATTGTATGGAGGCTT TGTTCCAAGGACCACTCCAGTATAAAGACTTGAAGATTGACATCAAGACGAGTCCC CCTCCTGAATGTATCAATGACTTGCTCCAAGCAGTTGACTCCCAGGAGGTGAGAGAT TACTGTGAGAAGAAGGGTTGGATAGTCAACATCACCAGCCAGGTTCAAACAGAAAG GAACATCAACAGGGCAATGACAATTCTACAAGCGGTGACAACCTTCGCCGCAGTGG CTGGAGTTGTCTATGTCATGTATAAACTGTTTGCTGGACACCAGGGAGCATACACTG GTTTACCAAACAAAAAACCCAACGTGCCCACCATTAGGACAGCAAAGGTACAAGGG CCAGGGTTCGATTACGCAGTGGCTATGGCTAAAAGAAACATTGTTACAGCAACTACT AGCAAGGGAGAGTTCACTATGTTAGGAGTCCACGACAACGTGGCTATTTTACCAAC CCACGCTTCACCTGGTGAAAGCATTGTGATCGATGGCAAAGAAGTGGAGATCTTGG ATGCCAAAGCGCTCGAAGATCAAGCAGGAACCAATCTTGAAATCACTATAATCACT CTAAAGAGAAATGAAAAGTTCAGAGACATTAGACCACATATACCTACTCAAATCAC TGAGACAAATGATGGAGTCTTGATCGTGAACACTAGCAAGTACCCCAATATGTATGT TCCTGTCGGTGCTGTGACTGAACAGGGATATCTAAATCTCGGTGGGCGCCAAACTGC TCGTACTCTAATGTACAACTTTCCAACCAGAGCAGGACAGTGTGGTGGAGTCATCAC ATGTACTGGGAAAGTCATCGGGATGCATGTTGGTGGGAACGGTTCACACGGGTTTG CAGCGGCCCTGAAGCGATCATACTTCACTCAGAGTCAAGGTGAAATCCAGTGGATG AGACCTTCGAAGGAAGTGGGATATCCAATCATAAATGCCCCGTCCAAAACCAAGCT TGAACCCAGTGCTTTCCACTATGTGTTTGAAGGGGTGAAGGAACCAGCAGTCCTCAC TAAAAACGATCCCAGGCTTAAGACAAACTTTGAGGAGGCAATTTTCTCCAAGTACGT GGGTAACAAAATTACTGAAGTGGATGAGCACATGAAAGAGGCAGTAGACCACTATG CTGGCCAGCTCATGTCACTAGACATCAACACAGAACAAATGTGCTTGGAGGATGCC ATGTATGGCACTGATGGTCTAGAAGCACTTGATTTGTCCACCAGTGCTGGCTACCCT TATGTAGCAATGGGAAAGAAGAAGAGAGATATCTTGAACAAACAAACCAGAGACA CTAAGGAAATGCAAAAACTGCTCGACACATATGGAATCAACCTCCCACTGGTGACT TATGTAAAGGATGAACTTAGATCCAAAACAAAGGTTGAGCAGGGGAAATCCAGATT AATTGAAGCTTCTAGTTTGAATGACTCAGTGGCAATGAGAATGGCTTTTGGGAACCT ATATGCTGCTTTTCACAAAAACCCAGGAGTGATAACAGGTTCAGCAGTAGGGTGCG ATCCAGATTTGTTTTGGAGCAAAATTCCGGTATTGATGGAAGAGAAGCTGTTTGCCT TTGACTACACAGGGTATGATGCATCTCTCAGCCCTGCTTGGTTCGAGGCACTAAAGA TGGTGCTTGAGAAAATCGGATTCGGAGACAGAGTTGACTACATCGACTACCTAAAC CACTCACACCACCTGTACAAGAATAAAACATACTGTGTCAAGGGCGGTATGCCATCT GGTTGCTCAGGCACTTCAATTTTTAACTCAATGATTAACAACTTGATTATCAGGACA CTCTTACTGAAAACCTACAAGGGCATAGATTTAGACCACCTAAAAATGATTGCCTAT GGTGATGATGTAATTGCTTCCTACCCCCATGAAGTTGACGCTAGTCTCCTAGCCCAA TCAGGAAAAGACTATGGACTAACTATGACTCCAGCTGACAAATCAGCTATATTTGA AACAGTCACATGGGAGAATGTAACATTCTTGAAGAGATTCTTCAGGGCAGACGAGA AATACCCATTTCTTATTCATCCAGTAATGCCAATGAAGGAAATTCATGAATCAATTA GATGGACAAAAGATCCTAGGAACACTCAGGATCACGTTCGCTCTCTGTGCCTATTAG CTTGGCACAATGGCGAAGAAGAATATAACAAATTCCTAGCTAAAATCAGGAGTGTG CCAATTGGAAGAGCTTTATTGCTCCCAGAGTACTCAACATTGTACCGCCGTTGGCTT GACTCATTTTAGTAACCCTACCTCAGTCGAATTGGATTGGGTCATACTGCTGTAGGG GTAAATTTTTCTTTAATTCGGAGAAAAAAAAAAA

Lerapolturev has tropism towards major components of the tumor and tumor microenvironment (TME), capable of infecting and promoting cytotoxicity of not only tumor cells, but of CD155-expressing infiltrating monocytes, macrophages, and dendritic cells (Freistadt et al. Virology. 195:798-803(1993)). Malignant cells of virtually all solid tumors, including bladder tumors, exhibit increased CD155 receptor expression (Luo et al. Front Oncol. 11:660273(2021); Takai et al. Nat Rev Mol Cell Biol. 9:603-15(2008); Chandramohan et al. Arch Pathol Lab Med. 141(12):1697-1704(2017); Liu et al. 11(15):5463-82(2019); Masson et al. Gut. 49:236-40(2001); Bevelacqua et al. Oncotarget. 3(8):882-92(2012); Carlsten et al. J Immunol. 183(8):4921-30(2009); Nishiwada et al. Anticancer Research. 35:2287-98(2015); Sun et al. International Immunopharmacology. 80:106198(2020); Zhang et al. Urol Oncol. 38(2):41(2020)). Recently it was shown that bladder cancer tumors demonstrate significantly upregulated CD155 expression which was found to be associated with poorer survival probability in human patients (Luo et al. Front Oncol. 11:660273(2021)). Bladder tumors with high CD155 expression also had significantly increased CD8+ T cell, neutrophil, macrophage, and dendritic cell tumor infiltration when compared with low CD155 expressing tumors (Luo et al. Front Oncol. 11:660273(2021)). While the presence of CD155 is sufficient for lerapolturev cell entry, it is not absolutely required for PVSRIPO replication. For example, lerapolturev infects antigen presenting cells (APCs)/dendritic cells (DCs) leading to the upregulation of antigen presentation (Brown et al. Science Translational Medicine. 9(408)(2017)) and the triggering of Type 1 interferon (IFN) inflammation in the TME (Brown et al. Science Translational Medicine. 9(408)(2017)).

Bladder Cancer Types

The methods described herein are used to treat a human patient with bladder cancer. In some embodiments, the bladder cancer is a resectable cisplatin-ineligible/refusal muscle invasive bladder cancer (MIBC). In some embodiments, the bladder cancer is a locally advanced or metastatic bladder cancer that has not progressed with first-line platinum-containing chemotherapy. In some embodiments, the bladder cancer is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk, non-muscle invasive bladder cancer (NMIBC) with carcinoma in situ (CIS) with or without papillary tumors who are ineligible for or have elected not to undergo

cystectomy. In some embodiments, the bladder cancer is a carcinoma in situ (CIS) of the urinary bladder. In some embodiments, the bladder cancer is a primary or recurrent stage Ta and/or T1 papillary bladder cancer tumors following transurethral resection (TUR). In some embodiments, the human patient is an adult with a low risk of disease recurrence and progression. In some embodiments, the human patient is an adult with a high risk of disease recurrence and progression. In some embodiments, the bladder cancer is a bladder cancer previously treated with PD-1 and/or PD-L1 inhibitor therapy.

In some embodiments, the bladder cancer is selected from a non-muscle invasive bladder cancer (NMIBC) or a muscle invasive bladder cancer (MIBC). In some embodiments, the patient has prior history of stage Ta, T1, or Tis urothelial carcinoma of the bladder. In some embodiments, the bladder tumors are comprised of up to 50% squamous or glandular differentiation. In some embodiments, the patient has documented tumor recurrence at cystoscopy where the bladder tumor is amenable to TURBT or cystectomy. In some embodiments, the patient has no history of variant bladder histologies selected from sarcomatoid, plasmacytoid, small cell or neuroendocrine, pure squamous cell carcinoma, pure adenocarcinoma, micropapillary, nested, lymphepithelioma-like, clear cell, or combinations thereof. In some embodiments, the patient has a history of variant bladder histologies selected from sarcomatoid, plasmacytoid, small cell or neuroendocrine, pure squamous cell carcinoma, pure adenocarcinoma, micropapillary, nested, lymphepithelioma-like, clear cell, or combinations thereof. In some embodiments, the patient has a measured or calculated (per institutional standard) creatinine clearance $\geq$ 30 ml/min. In some embodiments, the patient has a measured or calculated (per institutional standard) creatinine clearance $\geq$ 45 ml/min. In some embodiments, the patient has a GFR < 60 mL/min calculated per institutional standard. In some embodiments, the patient has a formalin-fixed paraffin-embedded tumor specimen with an associated pathology report documenting NMIBC. In some embodiments, the patient harbors bladder tumor lesions amenable to intratumoral injection. In some embodiments, the patient was previously exposed to intravesical agents selected from Bacillus Calmette-Guerin (BCG), mitomycin C, epirubicin, oncolytic viruses, investigational therapies. In some embodiments, the patient received no prior radiation to the pelvis. In some embodiments, the patient received prior radiation to the pelvis. In some embodiments, the patient received prior systemic therapy for bladder cancer. In some embodiments, the systemic therapy comprised administration of a PD-1/

PD-L1 inhibitor. In some embodiments, the patient has no history of vesicoureteric reflux or an indwelling urinary stent. In some embodiments, the patient has a history of vesicoureteric reflux or an indwelling urinary stent. In some embodiments, the patient has a history of stage T2 or higher bladder cancer. In some embodiments, the patient has no history of stage T2 or higher bladder cancer. In some embodiments, the patient has the ability to retain urine for 2 hours.

In some embodiments, the bladder cancer is a non-muscle invasive bladder cancer (NMIBC). In some embodiments, the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient is ineligible for cystectomy. In some embodiments, the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient who has elected not to undergo cystectomy. In some embodiments, the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient is ineligible for cystectomy. In some embodiments, the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient who has elected not to undergo cystectomy. In some embodiments, the human patient is an adult with a high risk of disease recurrence and progression. In some embodiments, the human patient is an adult with a low risk of disease recurrence and progression. In some embodiments, the subject is an adult selected from high risk, intermediate risk, or low risk of disease recurrence and progression. In some embodiments, the subject is an adult with a low risk of disease recurrence and progression. In some embodiments, the adult with low risk of disease recurrence and progression has low grade solitary Ta $\leq$ 3cm. In some embodiments, the adult with low risk of disease recurrence and progression has papillary urothelial neoplasm of low malignant potential. In some embodiments, the subject is an adult with an intermediate risk of disease recurrence and progression. In some embodiments, the adult with intermediate risk of disease recurrence and progression has low grade Ta that recurs within 1 year. In some embodiments, the adult with intermediate risk of disease recurrence and progression has solitary low grade TA > 3cm. In some embodiments, the adult with intermediate risk of disease recurrence and progression has multifocal low-grade Ta $\leq$3cm. In some embodiments, the adult with intermediate risk of disease recurrence and progression has low grade T1. In some embodiments, the subject is an adult with a high risk of disease recurrence and progression. In

some embodiments, the adult with high risk of disease recurrence and progression has high grade T1. In some embodiments, the adult with high risk of disease recurrence and progression has any recurrent high-grade Ta. In some embodiments, the adult with high risk of disease recurrence and progression has high grade Ta > 3cm or multifocal Ta. In some embodiments, the adult with high risk of disease recurrence and progression has any carcinoma in situ. In some embodiments, the adult with high risk of disease recurrence and progression has any BCG failure in a high-grade Ta. In some embodiments, the adult with high risk of disease recurrence and progression C has any variant histology. In some embodiments, the adult with high risk of disease recurrence and progression has any lymphovascular invasion. In some embodiments, the adult with high risk of disease recurrence and progression has any high grade prostatic urothelial involvement.

In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC). In some embodiments, the MIBC is a resectable cisplatin-ineligible/refusal MIBC. In some embodiments, the MIBC is a locally advanced or metastatic bladder cancer that has not progressed with first-line platinum-containing chemotherapy. In some embodiments, the MIBC is a unresectable, locally advanced tumor. In some embodiments, the unresectable, locally advanced MIBC tumor is selected from T4b, any N, ant T, N 2-3, M1, or combinations thereof.

Risk Stratification of Bladder Cancer Recurrence and/or Progression

NMIBC comprises noninvasive papillary carcinomas (Ta; 48%), submucosal invasive tumors (T1; 27%), carcinoma in situ (CIS; 2%) or some combination of these types (Boustead et al. BJU Int. 113(6):924-30(2014); Chang et al. J Urol. 196(4):1021-9(2016)); and subjects may be categorized as low-, intermediate-, or high-risk of disease recurrence and progression. In some embodiments, the subject has high risk, intermediate risk, or low risk of disease recurrence and progression. n some embodiments, the subject has a low risk of disease recurrence and progression . In some embodiments, the subject with low risk of disease recurrence and progression has low grade solitary Ta $\leq$ 3cm. In some embodiments, the subject with low risk of disease recurrence and progression has papillary urothelial neoplasm of low malignant potential. In some embodiments, the subject has an intermediate risk of disease recurrence and progression. In some embodiments, the subject with intermediate risk of disease recurrence and progression has low grade Ta that recurs within 1 year. In some embodiments, the subject with intermediate risk of

disease recurrence and progression has solitary low grade TA > 3cm. In some embodiments, the subject with intermediate risk of disease recurrence and progression has multifocal low-grade Ta ≤3cm. In some embodiments, the subject with intermediate risk of disease recurrence and progression has low grade T1. In some embodiments, the subject has a high risk of developing MIBC. In some embodiments, the subject with high risk of disease recurrence and progression has high grade T1. In some embodiments, the subject with high risk of disease recurrence and progression has any recurrent high-grade Ta. In some embodiments, the subject with high risk of disease recurrence and progression has high grade Ta > 3cm or multifocal Ta. In some embodiments, the subject with high risk of disease recurrence and progression has any carcinoma in situ. In some embodiments, the subject with high risk of disease recurrence and progression has any BCG failure in a high-grade Ta. In some embodiments, the subject with high risk of disease recurrence and progression has any variant histology. In some embodiments, the subject with high risk of disease recurrence and progression has lymphovascular invasion. In some embodiments, the subject with high risk of disease recurrence and progression has high grade prostatic urothelial involvement.

Previous Exposure to Bladder Cancer Treatments

In some embodiments, the subject to be treated has bladder cancer and was previously treated with an immunomodulatory intravesical agent, radiation therapy, a chemotherapeutic agent, an immune checkpoint inhibitor (ICI), or a combination thereof.

In some embodiments, the subject to be treated has bladder cancer and was previously treated with an immunomodulatory intravesical agent. In some embodiments, the immunomodulatory intravesical agent is selected from BCG, mitomycin C, epirubicin, or a combination thereof. In some embodiments, the immunomodulatory intravesical agent is BCG. In some embodiment, the subject is BCG-refractory. In some embodiments, the immunomodulatory intravesical agent is mitomycin C. In some embodiments, the immunomodulatory intravesical agent is epirubicin.

In some embodiments, the subject to be treated has bladder cancer and was previously treated with a chemotherapeutic agent. Previously administered chemotherapeutic agents include, but are not limited to, cisplatin, carboplatin, oxaliplatin, gemcitabine, doxorubicin, docetaxel,

methotrexate, vinblastine, cabazitaxel, or a combination thereof. In some embodiments, the chemotherapeutic agent is a platinum-based drug. In some embodiments, the chemotherapeutic agent is one or more platinum-based drug in combination with one or more non-platinum-based drug. In some embodiments, the platinum-based drug comprises cisplatin. In some embodiments, the platinum-based drug comprises carboplatin. In some embodiments, the platinum-based drug comprises oxaliplatin. In some embodiments, the chemotherapeutic agent is gemcitabine. In some embodiments, the chemotherapeutic agent is doxorubicin. In some embodiments, the chemotherapeutic agent is docetaxel. In some embodiments, the chemotherapeutic agent is methotrexate. In some embodiments, the chemotherapeutic agent is vinblastine. In some embodiments, the chemotherapeutic agent is cabazitaxel. In some embodiments, the chemotherapeutic agent is enfortumab vedotin.

In some embodiments, the subject to be treated has bladder cancer and was previously treated with radiation therapy. In some embodiments, the subject was previously treated with external-beam radiation therapy. In some embodiments, the subject was previously treated with intraoperative radiation therapy. In some embodiments, the subject was previously treated with image guided radiation therapy. In some embodiments, the subject was previously treated with intensity-modulated radiation therapy. In some embodiments, the subject was previously treated with x-ray beam radiation.

In some embodiments, the subject to be treated has bladder cancer and was previously treated with an ICI. In some embodiments, the ICI is selected from a PD-1 inhibitor or a PD-L1 inhibitor. In some embodiments, the ICI is an PD-1 inhibitor. In some embodiments, the PD-1 inhibitor is selected from pembrolizumab or nivolumab. In some embodiments, the PD-1 inhibitor is pembrolizumab. In some embodiments, the PD-1 inhibitor is nivolumab. In some embodiments, the ICI is a PD-L1 inhibitor. In some embodiments, the PD-L1 inhibitor is selected from atezolizumab, durvalumab, or avelumab. In some embodiments, the PD-L1 inhibitor is atezolizumab. In some embodiments, the PD-L1 inhibitor is durvalumab. In some embodiments, the PD-L1 inhibitor is avelumab.

In some embodiments, the subject to be treated has bladder cancer and was previously treated with a bladder cancer therapy selected from rogaratinib (BAY1163877), derazantinib (ARQ 087), tazemetostat (TAZVERIK®), cabozantinib (CABOMETYX®), sitravatinib

(MGCD516), adenovirus, a coxsackievirus, entinostat (SNDX-275/MS-275), epacadostat (INCB24360), CYT107, bempegaldesleukin (BEMPEG/NKTR-214), urelumab (BMS-663513), lirilumab (IPH2102), enfortumab vedotin (PADCEV®), oleclumab (MEDI9447), guadecitabine (SGI-110), olaparib (LYNPARZA®), or a combination thereof.

Induction Phase

As provided herein, a specifically-timed administration schedule of a chimeric poliovirus by intravesical instillation provides enhanced anti-bladder tumor efficacy and reduced tumor recurrence rate. For example, a chimeric poliovirus is administered to the subject at periodic intervals for the initiation of an immune effector cell response (i.e., an induction phase). In one embodiment, the chimeric poliovirus is administered in one or more induction phases.

In one aspect, the induction phase comprises one or more treatment cycles, wherein the treatment cycle comprises one or more weeks wherein a chimeric poliovirus is administered on the first day of each week. In some embodiments, the method is administered until disease progression or death. In some embodiments, the chimeric poliovirus comprises lerapolturev (also known as PVSRIPO). In some embodiments, the treatment cycle lasts 1-week, 2-weeks, 3-weeks, 4-weeks, 5-weeks, 6-weeks, 7-weeks, 8-weeks, 9-weeks, or 10-weeks. In some embodiments, the initiation of each of the one or more treatment cycles is 1-week, 2-weeks, 3-weeks, 4-weeks, 5 weeks, 6-weeks, 3-months, 6-months, or greater from each other. In some embodiments, each treatment cycle lasts 6-weeks. In some embodiments, each treatment cycle lasts 4-weeks. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase.

In another aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising one or more 6-week treatment cycles, wherein the one or more 6-week treatment cycles comprise administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, the treatment cycle lasts 1-week, 2-weeks, 3-weeks, 4-weeks, 5-weeks, 6-weeks, 7-weeks, 8-weeks, 9-weeks, or 10-weeks. In some embodiments, each of the one or more treatment cycles are administered 1-week, 2-weeks, 3-weeks, 4-weeks, 5 weeks, 6-

weeks, 3-months, 6-months, or greater from each other. In some embodiments, the induction cycle is repeated if no objective response rate (ORR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no complete response (CR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no partial response (PR) is exhibited by the patient. In some embodiments, the induction phase comprises 2 or more treatment cycles, for example, 2, 3, 4, or more than 5 treatment cycles. In some embodiments, the induction phase comprises between 2 and 5 treatment cycles. In some embodiments, the induction phase comprises at least 2, at least 3, at least 4, or 5 or more treatment cycles. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase.

In another aspect, provided herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises a single administration of an effective amount of a chimeric poliovirus intravesical instillation, wherein the chimeric poliovirus is administered within 24 hours of transurethral resection of the bladder tumor (TURBT). In some embodiments, the chimeric poliovirus is administered within 24 hours prior to a TURBT. In some embodiments, the chimeric poliovirus is administered within about 24 hours following a TURBT. In some embodiments, the chimeric poliovirus is lerapolturev. In some embodiments, the bladder cancer is non-muscle invasive bladder cancer (NMIBC). In some embodiments, the bladder cancer is muscle invasive bladder cancer (MIBC).

In another aspect, described herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising two 6-week treatment cycles, wherein each 6-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, the two 6-week treatment cycles are administered 1-week, 2-weeks, 3-weeks, 4-weeks, 5 weeks, 6-weeks, 3-months, 6-months, or greater from each other. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase.

In another aspect, the induction phase comprises one or more 4-week treatment cycles comprising administering to the patient an effective amount of a chimeric poliovirus by

intravesical instillation on the first day of each cycle. In some embodiments, the method is administered until disease progression or death. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, each of the one or more treatment cycles are administered 1-week, 2-weeks, 3-weeks, 4-weeks, 5 weeks, 6-weeks, 3-months, 6-months, or greater from each other. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase.

In another aspect, the induction phase comprises 4-week treatment cycles comprising administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each cycle, wherein the 4-week treatment cycle is repeated three times. In some embodiments, the chimeric poliovirus comprises lerapolturev. In some embodiments, each of the one or more treatment cycles are administered 1-week, 2-weeks, 3-weeks, 4-weeks, 5 weeks, 6-weeks, 3-months, 6-months, or greater from each other. In some embodiments, the induction phase is administered alone. In some embodiments, the induction phase is followed by a maintenance phase.

Maintenance Phase

In one embodiment, a chimeric poliovirus is administered to the subject at periodic intervals for the maintenance of the immune effector cell response (i.e., a maintenance phase). In one embodiment, the chimeric poliovirus is administered in one or more maintenance phases. In some embodiments, the maintenance phase is administered following an objective response rate (ORR) exhibited by the patient following the cessation of the induction phase. In some embodiments, the maintenance phase is administered following a complete response (CR) exhibited by the patient following the cessation of the induction phase. In some embodiments, the method is administered until disease progression or death. In some embodiments, the chimeric poliovirus comprises lerapolturev (also known as PVSRIPO). In some embodiments, the maintenance phase is administered alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase. In some embodiments, the maintenance phase is administered 1-week, 2-weeks, 3-weeks, 4-weeks, 1-month, 5-weeks, 6-

weeks, 2-months, 3-months, 4-months, 6-months, or greater following the cessation of the induction phase. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. As provided herein, each treatment cycle of the maintenance phase is administered at least 4 weeks apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase. In some embodiments, each treatment cycle of the maintenance phase is administered at least 4 weeks apart, at least 6 weeks apart, at least 8 weeks apart, at least 10 weeks apart, at least 12 weeks apart, or greater than 12 weeks apart. In some embodiments, each treatment cycle of the maintenance phase is administered at least 1 month apart, at least 2 months apart, at least 3 months apart, at least 4 months apart, at least 5 months apart, at least 6 months apart, or a combination thereof. In some embodiments, the subject is administered a maintenance phase alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase.

In one aspect, provided herein is a method of treating a human subject having bladder cancer, wherein the treatment comprises a maintenance phase, wherein the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of an effective dose of a chimeric poliovirus once a week by intravesical instillation, wherein each treatment cycle lasts 1-week, 2-weeks, 3-weeks, 4-weeks, or 6 weeks, and wherein each treatment cycle is administered 4 weeks apart, 6 weeks apart, 8 weeks apart, 10 weeks apart, 3 months apart, or 6 months apart. In some embodiments, the method is administered until disease progression or death. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. In some embodiments, the maintenance phase comprises 7 treatment cycles. In some embodiments, the maintenance

phase is administered alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase.

In another aspect, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 3 weeks, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the method is administered until disease progression or death. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. As provided herein, each treatment cycle of the maintenance phase is administered at least 4 weeks apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase. In some embodiments, each treatment cycle of the maintenance phase is administered at least 4 weeks apart, at least 6 weeks apart, at least 8 weeks apart, at least 10 weeks apart, at least 12 weeks apart, or greater than 12 weeks apart. In some embodiments, each treatment cycle of the maintenance phase is administered at least 1 month apart, at least 2 months apart, at least 3 months apart, at least 4 months apart, at least 5 months apart, at least 6 months apart, or a combination thereof. In some embodiments, the subject is administered a maintenance phase alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase.

In another aspect, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 2 weeks, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the method is administered until disease progression or death. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase

comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. As provided herein, each treatment cycle of the maintenance phase is administered at least 4 weeks apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase. In some embodiments, each treatment cycle of the maintenance phase is administered at least 4 weeks apart, at least 6 weeks apart, at least 8 weeks apart, at least 10 weeks apart, at least 12 weeks apart, or greater than 12 weeks apart. In some embodiments, each treatment cycle of the maintenance phase is administered at least 1 month apart, at least 2 months apart, at least 3 months apart, at least 4 months apart, at least 5 months apart, at least 6 months apart, or a combination thereof. In some embodiments, the subject is administered a maintenance phase alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase.

In another aspect, the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of a chimeric poliovirus once a week for 1 week, and wherein the chimeric poliovirus is administered to the bladder via intravesical instillation. In some embodiments, the method is administered until disease progression or death. In some embodiments, the maintenance phase comprises 2 or more treatment cycles, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10 treatment cycles. In some embodiments, the maintenance phase comprises between 2 and 10 treatment cycles. In some embodiments, the maintenance phase comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, or 11 or more treatment cycles. As provided herein, each treatment cycle of the maintenance phase is administered at least 4 weeks apart. In some embodiments, a treatment cycle of the maintenance phase is administered at the beginning of month 3, month 6, month 12, month 18, month 24, month 30, and month 36 following the start of the induction phase. In some embodiments, the initiation of each treatment cycle of the maintenance phase is administered at least 4 weeks apart, at least 6 weeks apart, at least 8 weeks apart, at least 10 weeks apart, at least 12 weeks apart, or greater than 12 weeks apart. In some embodiments, the initiation of each treatment cycle of the maintenance phase is administered at least 1 month apart, at least 2 months apart, at least 3 months apart, at least 4 months apart, at least 5 months apart, at least 6 months apart, or a combination thereof. In some embodiments, the subject is administered a maintenance

phase alone. In some embodiments, the maintenance phase is administered following the cessation of the induction phase.

Pre-Wash

In some embodiments, the chimeric poliovirus is administered via intravesical instillation following a pre-wash sequence. The pre-wash sequence may increase or enhance the transduction of the chimeric poliovirus. In some embodiments, the pre-wash results in the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the epithelium. In some embodiments, the pre-wash comprises a surfactant that disrupts the GAG layer, thereby providing access to the underlying epithelium of the bladder. In some embodiments, the surfactant is a mild polar surfactant. In some embodiments, the surfactant may be, but is not limited to, Tween-80, sodium dodecyl sulfate, or cetylpyridinium chloride. In some embodiments, the pre-wash comprises a calcium ion chelator that disrupts the GAG layer, thereby providing access to the underlying epithelium of the bladder. In some embodiments, the calcium ion chelator may be, for example, polycarbophil.

In some embodiments, the pre-wash sequence comprises one or more n-dodecyl-B-D-maltoside (DDM) washes and one or more saline washes. In some embodiments, the DDM pre-wash comprises between about 0.5% and about 10% DDM. In some embodiments, the pre-wash comprises between about 2% and about 6% of DDM. In some embodiments, the pre-wash comprises about 5% DDM. In some embodiments, a saline wash is administered prior to the DDM wash. In some embodiments, a saline wash is administered after the DDM wash. In some embodiments, a saline wash is administered prior to the DDM wash and then again after the saline wash. In some embodiments, the saline wash is administered and retained within the bladder for from about 2 minutes to 10 minutes. In some embodiments, the saline wash is administered and retained within the bladder for about 5 minutes. In some embodiments, the DDM wash is administered and retained within the bladder from about 2 minutes to about 25 minutes. In some embodiments, the DDM wash is administered and retained within the bladder for from about 10 minutes to about 20 minutes. In some embodiments, the DDM wash is administered and retained in the bladder for about 15 minutes +/- about 5 minutes. In some embodiments, the DDM wash is administered and retained in the bladder for about 5 minutes.

In some embodiments, prior to the administration of the chimeric poliovirus via intravesical instillation, the patient is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, ii) a third wash comprising about 100 ml saline. In some embodiments, prior to the administration of the chimeric poliovirus via intravesical instillation, the patient is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, iii) a third wash comprising about 100 ml saline, wherein each saline wash is administered and retained in the bladder for about 5 minutes and the DDM wash is administered and retained in the bladder for about 15 minutes +/- about 5 minutes.

Combinations

In some embodiments, the chimeric poliovirus is administered in combination with one or more anti-cancer therapies. In some embodiments, the anti-cancer therapy is selected from a chemotherapeutic agent, an immunomodulatory intravesical agent, radiation therapy, surgery, a therapeutic agent, or an immune checkpoint inhibitor (ICI). In some embodiments, the anti-cancer therapy is administered by intravesical instillation.

Chemotherapeutic Agents

In some embodiments, the methods described herein further include the administration of an effective amount of a chemotherapeutic agent. Suitable chemotherapeutic agents for use in the methods described herein include, but are not limited to, cisplatin, carboplatin, oxaliplatin, gemcitabine, mitomycin C, doxorubicin, epirubicin, docetaxel, methotrexate, vinblastine, cabazitaxel, or a combination thereof. In some embodiments, the chemotherapeutic agent is a platinum-based drug. In some embodiments, the chemotherapeutic agent is one or more platinum-based drug in combination with one or more non-platinum-based drug. In some embodiments, the platinum-based drug comprises cisplatin. In some embodiments, the platinum-based drug comprises carboplatin. In some embodiments, the platinum-based drug comprises oxaliplatin. In some embodiments, the chemotherapeutic agent is gemcitabine. In some embodiments, the chemotherapeutic agent is doxorubicin. n some embodiments, the chemotherapeutic agent is docetaxel. In some embodiments, the chemotherapeutic agent is methotrexate. In some

embodiments, the chemotherapeutic agent is vinblastine. In some embodiments, the chemotherapeutic agent is cabazitaxel. In some embodiments, the chemotherapeutic agent is administered by intravesical instillation.

Intravesical Agent

In some embodiments, the chimeric poliovirus is administered as descried herein in combination with an intravesical agent. In some embodiments, the intravesical agent is BCG. In some embodiments, the intravesical agent comprises an oncolytic virus other than a chimeric poliovirus.

Radiation Therapy

In some embodiments, the chimeric poliovirus is administered as described herein in combination with radiation therapy. In some embodiments, the radiation therapy is external-beam radiation therapy. In some embodiments, the radiation therapy is intraoperative radiation therapy. In some embodiments, the radiation therapy is image guided radiation therapy. In some embodiments, the radiation therapy is intensity-modulated radiation therapy. In some embodiments, the radiation therapy is x-ray beam radiation.

Surgery

In some embodiments, the chimeric poliovirus is administered as described herein prior to surgery to remove or reduce the bladder cancer mass. In some embodiments, the chimeric poliovirus is administered as described herein following surgery to remove or reduce the bladder cancer mass. In some embodiments, the surgery is a transurethral resection of bladder tumors (TURBT).

Therapeutic Agents

In some embodiments, the methods described herein further include the administration of an effective amount of a therapeutic agent. Suitable therapeutic agents for use in the methods described herein include, but are not limited to, a fibroblast growth factor receptor (FGFR) inhibitor, an EZH inhibitor, an RTK inhibitor, an oncolytic virus other than a chimeric poliovirus,

an IDO inhibitor, an antibody-drug conjugate, or a DNA moderator. In some embodiments, the therapeutic agent comprises an FGFR inhibitor. In some embodiments, the FGFR inhibitor is selected from rogaratinib (BAY1163877), derazantinib (ARQ 087), or a combination thereof. In some embodiments, the therapeutic agent comprises an (EZH) inhibitor. In some embodiments, the enhancer of zeste homolog 2 (EZH2) histone-lysine N-methyltransferase enzyme inhibitor comprises tazemetostat (TAZVERIK®). In some embodiments, the therapeutic agent comprises a receptor tyrosine kinase (RTK) inhibitor. In some embodiment, the RTK inhibitor is selected from cabozantinib (CABOMETYX®), sitravatinib (MGCD516), or a combination thereof. In some embodiments, the therapeutic agent is an oncolytic virus other than a chimeric poliovirus. In some embodiments, the oncolytic virus is selected from an adenovirus, a coxsackievirus, or a combination thereof. In some embodiments, the oncolytic virus other than a chimeric poliovirus is administered by intravesical instillation. In some embodiments, the therapeutic agent comprises an IDO inhibitor. In some embodiments, the indoleamine 2,3 dioxygenase (IDO) inhibitor is selected from entinostat (SNDX-275/MS-275), epacadostat (INCB24360), or a combination thereof. In some embodiments, the therapeutic agent is selected from CYT107, bempegaldesleukin (BEMPEG/NKTR-214), urelumab (BMS-663513), lirilumab (IPH2102), enfortumab vedotin (PADCEV®), or a combination thereof. In some embodiments, the therapeutic agent comprises a DNA moderator. In some embodiments, the DNA moderator comprises oleclumab (MEDI9447), guadecitabine (SGI-110), olaparib (LYNPARZA®), or a combination thereof.

Immune Checkpoint Inhibitors

In some embodiments, the methods described herein further include the administration of an effective amount of an immune checkpoint inhibitor (ICI) to a subject with bladder cancer. Suitable ICIs for use in the methods described herein include, but are not limited to, a programmed cell death -1 (PD-1) inhibitor, a programmed cell death-ligand 1 (PD-L1) inhibitor, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, a lymphocyte-activation gene 3 (LAG-3) inhibitor, a T-cell immunoglobulin mucin-3 (TIM-3) inhibitor, or a T cell immunoreceptor with Ig and ITIM domains (TIGIT) program death-ligand 2 (PD-L2), a V-domain Ig suppressor of T-cell activation (VISTA), B7-H3/CD276, indoleamine 2,3-dioxygenase (IDO), killer immunoglobulin-

like receptors (KIRs), carcinoembryonic antigen cell adhesion molecules (CEACAM) such as CEACAM-1, CEACAM-3, and CEACAM-5, sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15), a CD47 inhibitor, a CD39 inhibitor, or a B and T lymphocyte attenuator (BTLA) protein inhibitor, or a combination thereof.

PD-1 inhibitors

In some embodiments, the administered ICI is a PD-1 inhibitor that blocks the interaction of PD-1 and PD-L1 by binding to the PD-1 receptor, and in turn inhibits immune suppression. In some embodiments, the ICI is a PD-1 ICI selected from nivolumab (Opdivo®), pembrolizumab (Keytruda®), pidilizumab (Medivation), AMP-224 (Amplimmune); sasanlimab (PF-06801591; Pfizer), spartalizumab (PDR001; Novartis), cemiplimab (Libtayo®; REGN2810; Regeneron), retifanlimab (MGA012; MacroGenics), tislelizumab (BGB-A317; BeiGene), camrelizumab (SHR-1210; Jiangsu Hengrui Medicine Company and Incyte Corporation), CS1003 (Cstone Pharmaceuticals), and dostarlimab (TSR-042; Tesaro).

In some embodiments, the PD-1 inhibitor is nivolumab (Opdivo®) administered in an effective amount. In some embodiments, nivolumab is administered at 240 mg every 2 weeks or 480 mg every 4 weeks. In some embodiments, the PD-1 inhibitor is pembrolizumab (Keytruda®) administered in an effective amount. In some embodiments, pembrolizumab is administered at 200 mg every 3 weeks or 400 mg every 6 weeks. In some embodiments, the PD-1 inhibitor is cemiplimab (Libtayo®) administered in an effective amount. In some embodiments, cemiplimab is administered at 350 mg as an intravenous infusion over 30 minutes every 3 weeks.

PD-L1 inhibitors

In some embodiments, the immune checkpoint inhibitor (ICI) is a PD-L1 inhibitor that blocks the interaction of PD-1 and PD-L1 by binding to the PD-L1 receptor, and in turn inhibits immune suppression. PD-L1 inhibitors include, atezolizumab (Tecentriq®, Genentech), durvalumab (Imfinzi®, AstraZeneca); avelumab (Bavencio®; Merck), envafolimab (KN035; Alphamab), BMS-936559 (Bristol-Myers Squibb), lodapolimab (LY3300054; Eli Lilly), cosibelimab (CK-301; Checkpoint Therapeutics), sugemalimab (CS-1001; Cstone

Pharmaceuticals), adebrelimab (SHR-1316; Jiangsu HengRui Medicine), CBT-502 (CBT Pharma), and BGB-A333 (BeiGene).

In some embodiments, the ICI is the PD-L1 ICI atezolizumab (Tecentriq®) administered in an effective amount. In some embodiments, atezolizumab is administered at 840 mg every 2 weeks, 1200 mg every 3 weeks, or 1680 mg every 4 weeks. In some embodiments, atezolizumab is administered prior to chemotherapy. In another aspect of this embodiment, the ICI is durvalumab (Imfinzi®) administered in an effective amount. In some embodiments, durvalumab is administered at 10 mg/kg every 2 weeks or 1500 mg every 4 weeks for patients that weigh more than 30 kg and 10 mg/kg every 2 weeks for patients who weigh less than 30 kg. In another aspect of this embodiment, the ICI is avelumab (Bavencio®) administered in an effective amount. In some embodiments, avelumab is administered at 800 mg every 2 weeks. In yet another aspect of the embodiment, the ICI is KN035 (Alphamab) administered in an effective amount. An additional example of a PD-L1 ICI is BMS-936559 (Bristol-Myers Squibb).

T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT) Inhibitors

In some embodiments, the immune checkpoint inhibitor (ICI)is a T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). TIGIT is a promising new target for cancer immunotherapy. TIGIT is upregulated by immune cells, including activated T cells, natural killer cells, and regulatory T cells. TIGIT binds to two ligands, CD155 (PVR) and CD112 (PVRL2, nectin-2), that are expressed by tumor cells and antigen-presenting cells in the tumor microenvironment (Stanietsky et al., The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. Proc Natl Acad Sci U S A 2009; 106: 17858–63).

TIGIT (also called WUCAM, Vstm3, VSIG9) is a receptor of the Ig superfamily, which plays a critical role in limiting adaptive and innate immunity (Boles et al., A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. Eur J Immunol 2009; 39:695–703). TIGIT participates in a complex regulatory network involving multiple inhibitory receptors (e.g., CD96/TACTILE, CD112R/PVRIG), one competing costimulatory receptor (DNAM-1/CD226), and multiple ligands (e.g., CD155 (PVR/NECL-5), CD112 (Nectin-2/PVRL2) (Levin et al., Vstm3 is a member of the CD28 family and an important modulator of T-cell function. Eur J Immunol 2011; 41: 902–15; Bottino et al., Identification of PVR (CD155) and

nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. J Exp Med 2003; 198: 557–67; Seth et al., The murine pan T cell marker CD96 is an adhesion receptor for CD155 and nectin-1. Biochem Biophys Res Commun 2007; 364: 959–65; Zhu et al., Identification of CD112R as a novel checkpoint for human T cells. J Exp Med 2016; 213: 167–76).

TIGIT is expressed by activated CD8+ T and CD4+ T cells, natural killer (NK) cells, regulatory T cells (Tregs), and follicular T helper cells in humans (Joller et al., Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. J Immunol 2011; 186: 1338–42; Wu et al., Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice. Eur J Immunol 2016; 46: 1152–61). In sharp contrast with DNAM-1/CD226, TIGIT is weakly expressed by naive T cells. In cancer, TIGIT is co-expressed with PD-1 on tumor antigen-specific CD8+ T cells and CD8+ tumor-infiltrating lymphocytes (TILs) in mice and humans (Chauvin et al., Tigit and PD-1 impair tumor antigen-specific $CD8^+$ T cells in melanoma patients. J Clin Invest 2015; 125: 2046–58; Johnston et al., The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell 2014; 26 :923–37). It is also co-expressed with other inhibitory receptors, such as T cell immunoglobulin and mucin domain-containing molecule-3 (TIM-3) and lymphocyte activation gene 3 (LAG-3), on exhausted CD8+ T cell subsets in tumors (Chauvin et al., Tigit and PD-1 impair tumor antigen-specific $CD8^+$ T cells in melanoma patients. J Clin Invest 2015; 125: 2046–58; Johnston et al., The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. Cancer Cell 2014; 26 :923–37). Further, TIGIT is highly expressed by Tregs in peripheral blood mononuclear cells of healthy donors and patients with cancer and further upregulated in the TME (Joller et al., Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity 2014; 40: 569–81; Zhang et al., Genome-Wide DNA methylation analysis identifies hypomethylated genes regulated by FOXP3 in human regulatory T cells. Blood 2013; 122: 2823–36).

In some embodiments, the ICI is a TIGIT inhibitor that blocks the interaction of TIGIT and CD155 by binding to the TIGIT receptor, and in turn inhibits immune suppression. TIGIT inhibitors include, but are not limited to, Etigilimab (OMP-313M32; Oncomed Pharmaceuticals); Tiragolumab (MTIG7192A; RG6058; Roche/Genentech); Vibostolimab (MK-7684; Merck);

BMS-986207 (Bristol-Myers Squibb); AZD2936 (AstraZeneca); ASP8374 (Astellas/Potenza Therapeutics); Domvanalimab (AB154; Arcus Biosciences); IBI939 (Innovent Biologics); Ociperlimab (BGB-A1217; BeiGene); EOS884448 (iTeos Therapeutics); SEA-TGT (Seattle Genetics); COM902 (Compugen); MPH-313 (Mereo Biopharma); M6223 (EMD Serono); HLX53 (Shanghai Henlius Biotech); JS006 (Junshi Bio); mAb-7 (Stanwei Biotech); SHR-1708 (Hengrui Medicine); BAT6005 (Bio-Thera Solutions); GS02 (Suzhou Zelgen/Qilu Pharma); RXI-804 (Rxi Pharmaceuticals); NB6253 (Northern Biologics); ENUM009 (Enumreal Biomedical); CASC-674 (Cascadian Therapeutics); AJUD008 (AJUD Biopharma); and AGEN1777 (Agenus, Bristol-Myers Squibb)).

T-cell immunoglobulin and mucin domain 3 (TIM-3) inhibitors

In some embodiments, the immune checkpoint inhibitor (ICI) is a T-cell immunoglobulin and mucin domain 3 (TIM-3) inhibitor. TIM-3 is an immunoglobulin (Ig) and mucin domain-containing cell surface molecule that was originally discovered as a cell surface marker specific to interferon (IFN-γ) producing $CD4^+$ T helper 1 (Th1) and $CD8^+$ T cytotoxic 1 (Tc1) cells (Monney et al., Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* 2002; 415: 536–41). Tim-3 is coregulated and co-expressed along with other immune checkpoint receptors (PD-1, Lag-3, and TIGIT) on $CD4^+$ and $CD8^+$ T cells (Chihara et al., Induction and transcriptional regulation of the co-inhibitory gene module in T cells. *Nature* 2018; 558: 454–9; DeLong et al., Il-27 and TCR stimulation promote T cell expression of multiple inhibitory receptors. *ImmunoHorizons* 2019; 3: 13–25). In cancer, Tim-3 expression specifically marks the most dysfunctional or terminally exhausted subset of $CD8^+$ T cells (Fourcade et al., Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen–specific CD8+ T cell dysfunction in melanoma patients. *J Exp Med* 2010; 207: 2175–86; Sakuishi et al., Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. *J Exp Med* 2010; 207: 2187–94). Four ligands for Tim-3 have been identified: galectin-9, phosphatidylserine (PtdSer), high-mobility group protein B1 (HMGB1), and CEACAM-1.

In some embodiments, the ICI is a TIM-3 inhibitor that blocks the interaction of TIM-3 and galectin-9, phosphatidylserine (PtdSer), high-mobility group protein B1 (HMGB1), and/or CEACAM-1 by binding to the TIM-3 receptor, and in turn inhibits immune suppression. TIM-3

inhibitors include, but are not limited to, Sabatolimab (MGB453; Novartis Pharmaceuticals); Cobolimab (TSR-022; Tesaro/GSK); RG7769 (Genentech); MAS-825 (Novartis); Sym023 (Symphogen A/S); BGBA425 (BeiGene); R07121661 (Hoffmann-La Roche); LY3321367 (Eli Lilly and Company); INCAGN02390 (Incyte Corporation); BMS-986258 (ONO7807, Bristol-Myers Squibb); AZD7789 (AstraZeneca); TQB2618 (Chia Tai Tianqing Pharmaceutical Group Co., Ltd.); and NB002 (Neologics Bioscience).

Lymphocyte activation gene-3 (LAG-3) inhibitors

In some embodiments, the immune checkpoint inhibitor (ICI) is a LAG-3 inhibitor. LAG-3 (CD223) is encoded by the LAG-3 gene. LAG-3 is a member of the immunoglobulin superfamily (IgSF) and exerts a wide variety of biologic impacts on T cell function (Triebel et al., LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med* 1990; 171: 1393–405). LAG-3 is expressed on cell membranes of natural killer cells (NK), B cells, tumor-infiltrating lymphocytes (TIL), a subset of T cells, and dendritic cells (DC) (Triebel et al., LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med* 1990; 171: 1393–405); Kisielow et al., Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells. *Eur J Immunol* 2005; 35: 2081–8; Grosso et al., LAG-3 regulates CD8+ T cell accumulation and effector function in murine self- and tumor-tolerance systems. *J Clin Invest* 2007; 117: 3383–92; Workman et al., LAG-3 regulates plasmacytoid dendritic cell homeostasis. *J Immunol* 2009; 182: 1885–91; Andreae et al., Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223). *J Immunol* 2002; 168: 3874–80). The LAG-3 protein binds a nonholomorphic region of major histocompatibility complex 2 (MHC class II) with greater affinity than CD 4 (Baixeras et al., Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J Exp Med* 1992; 176: 327–37). LAG-3 is one of the various immune-checkpoint receptors that are coordinately upregulated on both regulatory T cells (Tregs) and anergic T cells, and the simultaneous blockade of these receptors can result in an enhanced reversal of this anergic state relative to the blockade of one receptor alone (Grosso et al., Functionally distinct LAG-3 and PD-1 subsets on activated and chronically stimulated CD8 T cells. *J Immunol* 2009; 182: 6659–69). The LAG-3/MHC class II molecule interaction leads to the downregulation of $CD4^+$ Ag-specific T cell clone proliferation and cytokine secretion (Huard

et al., T cell major histocompatibility complex class II molecules down-regulate $CD4^+$ T cell clone responses following LAG-3 binding. *Eur J Immunol* 1996; 26: 1180–6).

In some embodiments, the checkpoint inhibitor is a LAG-3 inhibitor that blocks the interaction of LAG-3 with major histocompatibility complex 2 (MHC class II) by binding to the LAG-3 receptor, and in turn inhibits immune suppression. LAG-3 inhibitors include, but are not limited to, relatlimab (BMS 986016/Ono 4482; Bristol-Myers Squibb); tebotelimab (MGD013; Macrogenics); LAG525 (Immutep, Novartis); TSR-033 (Tesaro, GlaxoSmithKline); Eftilagimod alpha (IMP321, Immutep); REGN3767 (Regeneron); INCAGN02385 (Incyte); RO7247669 (Hoffman-LaRoche); Favezelimab (Merck Sharp & Dohme); CB213 (Crescendo Biologics); FS118 (F-star Therapeutics); SYM022 (Symphogen); GSK2831781 (GlaxoSmithKline); IBI323 (Innovent Biologics (Suzhou) Co. Ltd.); EMB-02 (Shanghai EpimAb Biotherapeutics Co., Ltd.); SNA03 (Microbio Group); and AVA021 (Avacta).

Additional Immune Checkpoint Inhibitors

In some embodiments, the patient is administered a B7-H3/CD276 immune checkpoint inhibitor (ICI) such as enoblituzumab (MGA217, Macrogenics) MGD009 (Macrogenics), 131I-8H9/omburtamab (Y-mabs), and I-8H9/omburtamab (Y-mabs), an indoleamine 2,3-dioxygenase (IDO) ICI such as Indoximod and INCB024360, a killer immunoglobulin-like receptors (KIRs) ICI such as Lirilumab (BMS-986015), a carcinoembryonic antigen cell adhesion molecule (CEACAM) inhibitor (e.g., CEACAM-1, -3 and/or -5). Exemplary anti-CEACAM-1 antibodies are described in WO 2010/125571, WO 2013/082366 and WO 2014/022332, e.g., a monoclonal antibody 34B1, 26H7, and 5F4; or a recombinant form thereof, as described in, e.g., US 2004/0047858, U.S. Pat. No. 7,132,255 and WO 99/052552. In other embodiments, the anti-CEACAM antibody binds to CEACAM-5 as described in, e.g., Zheng et al. PLoS One. 2010 September 2; 5(9). pii: e12529 (DOI:10:1371/journal.pone.0021146), or cross-reacts with CEACAM-1 and CEACAM-5 as described in, e.g., WO 2013/054331 and US 2014/0271618.

In some embodiments, the patient is administered an ICI directed to CD47, including, but not limited to, Hu5F9-G4 (Stanford University/Forty Seven), TI-061 (Arch Oncology), TTI-622 (Trillum Therapeutics), TTI-621 (Trillum Therapeutics), SRF231 (Surface Oncology), SHR-1603 (Hengrui), OSE-172 (Boehringer Ingelheim/OSE Immunotherapeutics), NI-1701 (Novimmune

TG Therapeutics), IBI188 (Innovent Biologics); CC-95251 (Celgene), CC-90002 (Celgene/Inibrx), AO-176 (Arch Oncology), ALX148 (ALX Oncology), IMM01 (ImmuneOnco Biopharma), IMM2504 (ImmuneOnco Biopharma), IMM2502 (ImmuneOnco Biopharma), IMM03 (ImmuneOnco Biopharma), IMC-002 (ImmuneOncia Therapeutics), IBI322 (Innovent Biologics), HMBD-004B (Hummingbird Bioscience), HMBD-004A (Hummingbird Bioscience), HLX24 (Henlius), FSI-189 (Forty Seven), DSP107 (KAHR Medical), CTX-5861 (Compass Therapeutics), BAT6004 (Bio-Thera), AUR-105 (Aurigene), AUR-104 (Aurigene), ANTI-CD47 (Biocad), ABP-500 (Abpro), ABP-160 (Abpro), TJC4 (I-MAB Biopharma), TJC4-CK (I-MAB Biopharma), SY102 (Saiyuan), SL-172154 (Shattuck Labs), PSTx-23 (Paradigm Shift Therapeutics), PDL1/ CD47BsAb (Hanmi Pharmaceuticals), NI-1801 (Novimmune), MBT-001 (Morphiex), LYN00301 (LynkCell), and BH-29xx (Beijing Hanmi).

In some embodiments, the ICI is an inhibitor directed to CD39, including, but not limited to TTX-030 (Tizona Therapeutics), IPH5201 (Innate Pharma/AstraZeneca), SRF-617 (Surface Oncology), ES002 (Elpisciences), 9-8B (Igenica), and an antisense oligonucleotide (Secarna)

In some embodiments, the ICI is an inhibitor directed to B and T lymphocyte attenuator molecule (BTLA), for example as described in Zhang et al., Monoclonal antibodies to B and T lymphocyte attenuator (BTLA) have no effect on in vitro B cell proliferation and act to inhibit in vitro T cell proliferation when presented in a cis, but not trans, format relative to the activating stimulus, Clin Exp Immunol. 2011 Jan; 163(1):77–87, and TAB004/JS004 (Junshi Biosciences).

In some embodiments, the ICI is a sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) inhibitor, including, but not limited to, NC318 (an anti-Siglec-15 mAb).

In some embodiments, the ICI is opdualag, a combination of the LAG-3 checkpoint inhibitor relatimab and the PD-1 inhibitor nivolumab.

Pharmaceutical Compositions and Dosage Forms

The chimeric poliovirus for administration in the methods described herein can be administered, for example, as a pharmaceutical composition that includes an effective amount of the chimeric poliovirus for a patient, typically a human, in need of such treatment in a pharmaceutically acceptable carrier.

Carriers include excipients and diluents and should be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The carrier can be inert or it can possess pharmaceutical benefits of its own. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound.

Classes of carriers include, but are not limited to adjuvants, binders, buffering agents, coloring agents, diluents, disintegrants, excipients, emulsifiers, flavorants, gels, glidents, lubricants, preservatives, stabilizers, surfactants, solubilizer, tableting agents, wetting agents or solidifying material.

Exemplary pharmaceutically acceptable carriers include sugars, starches, celluloses, powdered tragacanth, malt, gelatin; talc, petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers and vegetable oils. Optional active agents may be included in a pharmaceutical composition, which do not substantially interfere with the activity of the compound of the present invention.

Some excipients include, but are not limited, to liquids such as water, saline, glycerol, polyethylene glycol, hyaluronic acid, ethanol, and the like.

Additionally, auxiliary substances, such as wetting or emulsifying agents, biological buffering substances, surfactants, and the like, can be present in such vehicles. A biological buffer can be any solution which is pharmacologically acceptable, and which provides the formulation with the desired pH, i.e., a pH in the physiologically acceptable range. Examples of buffer solutions include saline, phosphate buffered saline, Tris buffered saline, Hank's buffered saline, and the like.

In yet another embodiment provided is the use of permeation enhancer excipients including polymers such as: polycations (chitosan and its quaternary ammonium derivatives, poly-L-arginine, aminated gelatin); polyanions (N-carboxymethyl chitosan, poly-acrylic acid); and, thiolated polymers (carboxymethyl cellulose-cysteine, polycarbophil-cysteine, chitosan-thiobutylamidine, chitosan-thioglycolic acid, chitosan-glutathione conjugates).

In certain embodiments the excipient is selected from butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol,

methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

Typically, sterile injectable suspensions are formulated according to techniques known in the art using suitable carriers, dispersing or wetting agents and suspending agents. The sterile injectable formulation can also be a sterile injectable solution or a suspension in a acceptably nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils, fatty esters or polyols are conventionally employed as solvents or suspending media. In addition, parenteral administration can involve the use of a slow release or sustained release system such that a constant level of dosage is maintained.

Injectable formulations can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solubilization or suspension in liquid prior to injection, or as emulsions. Typically, sterile injectable suspensions are formulated according to techniques known in the art using suitable carriers, dispersing or wetting agents and suspending agents. The sterile injectable formulation can also be a sterile injectable solution or a suspension in an acceptably nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils, fatty esters or polyols are conventionally employed as solvents or suspending media.

The pharmaceutical composition comprising the chimeric poliovirus is administered in a therapeutically effective amount by any desired mode of administration, but is typically administered as an intravesical instillation into the bladder, or alternatively, intratumor injection or infusion, or alternatively, topically applies to a tumor lesion. Administration via intravesical instillation is generally known in the art. Intravesical instillation is generally performed by inserting a urinary catheter to first fully drain the bladder. A suspension comprising for example a chimeric poliovirus (e.g. 30 mL suspension comprising lerapolturev) is subsequently instilled into the bladder via an adapter with the catheter. The instillation generally lasts between about 3

minutes to about 5 minutes, wherein after the instillation is finished, the catheter is removed. In some embodiments, the patient is mobilized to allow the instilled suspension comprising chimeric poliovirus to contact all inner surface area of the bladder. In some embodiments, the patient is mobilized for a period of 2 hours. In some embodiments, the patient is mobilized comprising rotating between positions selected from prone, supine, left lateral, or right lateral.

Administration via intratumoral injection can involve introducing the formulations of the disclosure into one or more tumor lesions of a patient through a needle or a catheter, propelled by a sterile syringe or some other mechanical device such as a continuous infusion system. A formulation provided by the disclosure can be administered using a syringe, injector, pump, or any other device recognized in the art for parenteral administration.

In some embodiments, when the chimeric poliovirus is lerapolturev, lerapolturev is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS). Lerapolturev can be provided in sterile, single use glass vials with a flip off top containing approximately 0.5 mL of stock lerapolturev (for example, about 2.0×10^9 to about 1.0×10^{10} $TCID_{50}$).

In certain embodiments, as provided herein the chimeric poliovirus may be administered in a pharmaceutical composition comprising a detergent. As provided herein, useful detergents include, but are not limited to, n-dodecyl-B-D-maltoside (DDM), Tween-80, or SIM3. DDM is a maltoside-based non-ionic detergent with a hydrophilic maltose head and a hydrophobic long chain alkyl tail. It is considered a gentle detergent that is more efficient than other detergents, such as NP-40. A primary attribute of DDM is its capability to extract hydrophobic proteins while maintaining the solution-phase protein conformation. DDM also allows the protein to be reformed following denaturation. Tween-80 is a nonionic surfactant and emulsifier often used in foods and cosmetics. It is derived from polyethoxylated sorbitan and oleic acid. Examples of surfactants include, for example, polyoxyethylene glycol, polyoxypropylene glycol, decyl glucoside, lauryl glucoside, octyl glucoside, polyoxyethylene glycol octylphenol, Triton X-100, glycerol alkyl ester, glyceryl laurate, cocamide MEA, cocamide DEA, dodecyldimethylamine oxide, and poloxamers. Examples of poloxamers include, poloxamers 188, 237, 338 and 407. These poloxamers are available under the trade name Pluronic® (available from BASF, Mount Olive, N.J.) and correspond to Pluronic® F-68, F-87, F-108 and F-127, respectively. Poloxamer 188

(corresponding to Pluronic® F-68) is a block copolymer with an average molecular mass of about 7,000 to about 10,000 Da, or about 8,000 to about 9,000 Da, or about 8,400 Da. Poloxamer 237 (corresponding to Pluronic® F-87) is a block copolymer with an average molecular mass of about 6,000 to about 9,000 Da, or about 6,500 to about 8,000 Da, or about 7,700 Da. Poloxamer 338 (corresponding to Pluronic® F-108) is a block copolymer with an average molecular mass of about 12,000 to about 18,000 Da, or about 13,000 to about 15,000 Da, or about 14,600 Da. Poloxamer 407 (corresponding to Pluronic® F-127) is a polyoxyethylene-polyoxypropylene triblock copolymer in a ratio of between about E101 P56 E101 to about E106 P70 E106, or about E101 P56E101, or about E106 P70 E106, with an average molecular mass of about 10,000 to about 15,000 Da, or about 12,000 to about 14,000 Da, or about 12,000 to about 13,000 Da, or about 12,600 Da. Additional examples of surfactants that can be used in the invention include, but are not limited to, polyvinyl alcohol (which can be hydrolyzed polyvinyl acetate), polyvinyl acetate, Vitamin E-TPGS, poloxamers, cholic acid sodium salt, dioctyl sulfosuccinate sodium, hexadecyltrimethyl ammonium bromide, saponin, TWEEN® 20, TWEEN® 80, sugar esters, Triton X series, L-a-phosphatidylcholine (PC), 1 ,2-dipalmitoylphosphatidycholine (DPPC), oleic acid, sorbitan trioleate, sorbitan mono-oleate, sorbitan monolaurate, polyoxyethylene (20) sorbitan monolaurate, polyoxyethylene (20) sorbitan monooleate, natural lecithin, oleyl polyoxyethylene (2) ether, stearyl polyoxyethylene (2) ether, lauryl polyoxyethylene (4) ether, block copolymers of oxyethylene and oxypropylene, synthetic lecithin, diethylene glycol dioleate, tetrahydrofurfuryl oleate, ethyl oleate, isopropyl myristate, glyceryl monooleate, glyceryl monostearate, glyceryl monoricinoleate, cetyl alcohol, stearyl alcohol, cetylpyridinium chloride, benzalkonium chloride, olive oil, glyceryl monolaurate, corn oil, cotton seed oil, sunflower seed oil, lecithin, oleic acid, and sorbitan trioleate. In some embodiments, the detergent is administered by intravesical instillation. In some embodiments, the administration of detergent increases cellular viral intake. In some embodiments, the detergent is administered one or more times as a pre-wash. In some embodiments, the detergent is administered with a chimeric poliovirus by intravesical instillation. In some embodiments, the pharmaceutical composition described herein further comprises detergent. In some embodiments, the detergent comprises Tween-80. In some embodiments, the detergent comprises DDM. In some embodiments, the detergent comprises SIM-3. In some embodiments, the detergent is administered by intravesical instillation in a solution at a

concentration between at about 0.1% and at about 1.0%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 0.1%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 0.5%. In some embodiments, the detergent is administered by intravesical instillation in a solution at a concentration of at about 1.0%.

In certain embodiments, as provided herein the chimeric poliovirus may be administered in a pharmaceutical composition comprising a hydrogel. In some embodiments, the hydrogel is a thermal hydrogel. In some embodiments, the hydrogel is a reverse-thermal hydrogel, wherein the hydrogel is a liquid when cold and converts to a gel form at body temperature. Reverse-thermal hydrogels are known in the art and include, for example, RTGel™ manufactured by Urogen Pharma.

Improved Patient Outcomes

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides enhanced objective response rate (ORR) in the patients receiving the treatment. ORR is generally defined as the proportion of patients achieving a complete response (CR) or partial response (PR) per RECIST 1.1. Examples of an objective response (OR) includes a complete response (CR), which is the disappearance of all signs of the tumor in response to treatment and a partial response (PR), which is a decrease in the size of a tumor in response to treatment. In some embodiments, the OR is a CR. In some embodiments, the OR is a PR. The ORR is an important parameter to demonstrate the efficacy of a treatment and it serves as a primary or secondary endpoint in clinical trials. Methods of assessing ORR are well known in the art and include, for example RECIST v1.1 (Eisenhauer et al. Eur J Cancer. 45:228-47(2009)) and World Health Organization (WHO) (World Health Organization. WHO Handbook for Reporting Results of Cancer Treatment. World Health Organization Offset Publication No. 48; Geneva (Switzerland), 1979). In some embodiments, the induction cycle is repeated if no objective response rate (ORR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no complete response (CR) is exhibited by the patient. In some embodiments, the induction cycle is repeated if no partial response (PR) is exhibited by the patient. In some embodiments, the maintenance phase is administered following an objective response rate

(ORR) exhibited by the patient following the cessation of the induction phase. In some embodiments, the maintenance phase is administered following a complete response (CR) exhibited by the patient following the cessation of the induction phase.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides enhanced clinical benefit rate (CBR) in the patients receiving the treatment. CBR is generally defined as the proportion of patients with CR (for any duration), PR (for any duration) or SD ($\geq$ 6 months) per RECIST 1.1.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides lengthened duration of response (DOR) in the patients receiving the treatment. DOR is generally defined as the time from OR (per RECIST 1.1) until unequivocal disease progression or death, whichever occurs first.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides lengthened progression-free survival (PFS) in the patients receiving the treatment. PFS is generally defined as the time (measured in number of months) from the date of assigning patient ID number until date of documented radiologic disease progression per RECIST 1.1 or death due to any cause, whichever come first.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides enhanced overall survival (OS) in the patients receiving the treatment. OS is generally defined as the time (measured in number of months) from the date of assigning patient ID number until death due to any cause.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides alterations from baseline in immune function markers in blood samples and/or tissue, as data permits, in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides alterations from baseline in tumor biomarkers in tumor samples, as data permits, in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus provides alterations in genetic markers in tumor biopsies and/or blood samples that correlate with response in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus provides alterations in cytologic markers in tumor biopsies and/or blood samples that correlate with response in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus provides alterations in histologic markers in tumor biopsies and/or blood samples that correlate with response in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus provides alterations in molecular markers in tumor biopsies and/or blood samples that correlate with response in the patients receiving the treatment.

In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides reduced recurrence rate of bladder tumor formation. In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides decreased need for bladder tumor resection surgeries including but not limited to transurethral resection of bladder tumor (TURBT) or cystectomy. In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides decreased frequency of bladder tumor resection surgeries including but not limited to TURBT or cystectomy. In some embodiments, a treatment regimen comprising intravesical instillation administration of a chimeric poliovirus described herein provides lengthened relapse-free survival (RFS).

EXAMPLES

The claimed invention is further described by way of the following non-limiting examples. Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art, in view of the above disclosure and following experimental exemplification, included by way of illustration and not limitation, and with reference to the attached figures.

Example 1. Chemical compatibility of chimeric poliovirus lerapolturev

The purpose of this study was to characterize the potency of lerapolturev after exposure to various insults including urine, saliva, detergent, and various pH levels. The positive control sample for each condition was lerapolturev with regular growth media for the entire length of the experiment. The negative control sample for each condition was formulation buffer with regular growth media. The mock sample for each condition was formulation buffer with adjusted growth media (e.g., Tween-80, DDM, pH, Urine, Saliva) while the experimental sample(s) were lerapolturev with adjusted growth media (Table 3). Total incubation time lasted 2 hours.

Post-incubation, lerapolturev was diluted to multiplicities of infectivity (MOI) of 20, 6.6, or 2.0 using regular growth media. Dilutions were then added to U-87 MG (U87) human primary glioblastoma cell seeded plates in triplicate and incubated for 42 ± 4 hours. An MTS assay cytotoxicity assay was conducted wherein MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium)/ phenazine methosulfate (PMS) reagent was added to samples which is reduced by NAD(P)H-dependent cellular oxidoreductase enzymes to produce formazan, a compound with a max absorbance value of 490 nm in PBS. The amount of formazan product released is proportional to the number of viable cells present in each sample and inversely related to the degree of lerapolturev infectivity (i.e., low absorbance ~ low cell viability/greater lerapolturev infectivity; high absorbance ~ high cell viability/lower lerapolturev infectivity). The sample absorbance values at 490 nM were analyzed on a Spectramax ID5 multi-mode microplate reader.

Table 3. U87 lerapolturev Infectivity Assay

Tween-80	DDM	pH	Urine	Saliva
Growth Media – Tween-80 1.0%	Growth Media – DDM 1.0%	Growth Media – pH3	Growth Media – Urine 100%	Growth Media – Saliva 100%
Growth Media – Tween-80 0.5%	Growth Media – DDM 0.5%	Growth Media – pH7	Growth Media – Urine 55.6%	Growth Media – Saliva 55.6%
Growth Media – Tween-80 0.1%	Growth Media – DDM 0.1%	Growth Media – pH10*	Growth Media – Urine 37%	Growth Media – Saliva 37%
			Growth Media – Urine 11.1%	Growth Media – Saliva 11.1%
n=8 samples	n=8 samples	n=8 samples	n=10 samples	n=10 samples

*sample affected lerapolturev infectivity (pH 10)

Final Tween-80 concentration values in the cell media were 1%, 0.5%, and 0.1%. Triplicate values were obtained for each experimental condition (n=3 test samples/condition, n=3 mock samples/condition). Two samples of growth media positive control and negative control were tested for each condition. A 2-hour contact period with Tween-80 ranging from 0.1%-1.0% concentration had no impact on lerapolturev infectivity (FIG. 1A) at any MOI.

Final DDM concentration values in the cell media were 1%, 0.5%, and 0.1%. Triplicate values were obtained for each experimental condition (n=3 test samples/condition, n=3 mock samples/condition). A 2-hour contact period with DDM ranging from 0.1%-1.0% concentration had no impact on lerapolturev infectivity (FIG. 1B).

Final media pH values were 3, 7, and 10. Triplicate values were obtained for each experimental condition (n=3 test samples/condition, n=3 mock samples/condition). While pH 3 and pH 7 conditions had no effect on lerapolturev infectivity, pH 10 was the only condition to impact the ability of lerapolturev to infect U87 cells (FIG. 1C). At all MOI values, media at pH10 had absorbance values comparable with negative control, suggesting that solutions at pH 10 inhibit lerapolturev infectivity.

Urine concentration values in the cell media were 100%, 55.6%, 37% and 11.1%. Quadruplicate values were obtained for each experimental condition (n=4 test samples/condition, n=4 mock samples/condition). A 2-hour contact period with urine in the cell media ranging from 10%-90% concentration had no impact on lerapolturev infectivity (FIG. 1D).

Saliva concentration values in the cell media were 100%, 55.6%, 37% and 11.1%. Quadruplicate values were obtained for each experimental condition (n=4 test samples/condition, n=4 mock samples/condition). A 2-hour contact period with saliva in the cell media ranging from 11.1%-100% concentration had no impact on lerapolturev infectivity (FIG. 1E).

The only chemical condition that was observed to affect the cellular infectivity of lerapolturev was growth media at pH 10. This suggests that lerapolturev can be administered in a wide range of chemical compositions.

Lerapolturev infection stimulates a robust Type-I/III interferon (IFN)-dominant immune response and ultimately, anticancer T cell activation and recruitment (Brown et al. Sci Transl Med. 9:eaan4220(2017); Mosaheb et al. Nat Commun. 11:524(2020); Holl et al. Oncotarget. 7:79828-

41(2016)). An IFN-beta (IFN-β) enzyme-linked-immunosorbent assay (ELISA) immodulation assay was developed to examine the effect of various chemical insults including detergent, pH, urine, and saliva on the potency of lerapolturev infection and cellular response. Various growth media were incubated with lerapolturev for 2 hr at 37°C and adjusted to proper MOI (2.0, 6.6, 20). The amount of IFN-β secreted by A375 melanoma cells (ATCC, CR-1619) infected with lerapolturev was assayed by harvesting supernatant samples 48 hours post infection using human IFN-β Quantikine ELISA Kit (R&D Systems, DIFNB0). System suitability and assay variability were evaluated with all tested ELISA plates using ELISA positive and negative controls. The positive controls were within the acceptable criteria recommended by the vendor (Table 4).

Table 4. System suitability values

	IFN-β pg/mL	CV% (N=3)	Mean Result	SD	CV%
Positive High (R&D QC243) 196-370 pg/mL	280.287	11.198	281.7	1.9	0.68%
	283.014	5.778			
Positive Medium (R&D QC243) 121-198 pg/mL	143.493	3.151	146.2	5.8	3.96%
	152.671	0.579			
	145.358	2.416			
	138.434	5.474			
	151.052	2.758			
Positive Low (R&D QC243) 41.5-71.3 pg/mL	49.846	3.354	48.4	2.2	4.60%
	47.456	3.096			
	48.41	0.291			
	50.259	0.667			
	47.906	7.269			
	44.224	7.649			
	50.764	1.522			

Assay variability was evaluated using three different lerapolturev lots with multiple MOI (2, 6.6, 20). Coefficient of variation (CV%) ranged from 28% to 111% from plate to plate. Intra-plate variability was significantly lower for MOI 6.6 and MOI 20. This suitability and assay variability assay demonstrated that MOI 6.6 or 20 had consistently high IFN-β response and were the optimal concentrations for immunomodulation testing (Table 5).

Table 5. Assay variability values

	IFN-β pg/mL	CV% (N=3)	Mean (All)	SD	CV% (All)
GM Positive Control MOI 2	20.071	19.263	6.3	7.0	111%
	4.319	14.654			
	3.208	33.726			
	3.691	91.5			
	0.467	61.889			
	6.063	0			
GM Positive Control MOI 6.6	137.046	65.867	109	30	28%
	154.884	3.798			
	90.487	6.018			
	91.118	13.804			
	79.366	9.056			
	98.88	25.868			
GM Positive Control MO I20	535.358	3.949	315	225	72%
	568.824	7.226			
	106.603	8.145			
	121.884	22.09			
	109.052	11.074			
	449.344	6.075			

Following this, the chemical compatibility of lerapolturev immunomodulation activity (IFN-β secretion) was examined. Some moderate to severe inhibition of IFN-β secretion was seen with higher concentrations of detergents Tween-80 and DDM. Tween-80 had no effect on IFN-β release at 0.1% level, with only moderate inhibition between the 0.5%-1% concentration range (FIG. 2A). Meanwhile, DDM exhibited strong inhibitory effect on IFN-β release (lerapolturev immunomodulation activity) at 0.5%-1% level, although no impact on IFN-β levels was observed at 0.1% DDM concentration (FIG. 2B). This suggests that solutions comprising 0.1% DDM do not substantially inhibit chimeric poliovirus-mediated IFN-β signaling. Solutions at pH3 showed moderate inhibition of IFN-β secretion, whereas pH 7 had no effect on IFN-β levels (FIG. 2C). In comparison, pH 10 conditions eliminated IFN-β response. High concentrations of urine (100%) had a strong inhibitory effect on IFN-β release (lerapolturev immunomodulation activity) (FIG. 2D). Urine at 56%, 37%, or 11% concentration levels had moderate to no effect on IFN-β release. High concentrations of Saliva (56, 100%) had strong inhibitory effects on IFN-β release (FIG. 2E), although this inhibition was only seen at MOI 20 conditions. Low concentrations of saliva (37%,

11%) had moderate to no effect on IFN-β release. Taken together, these results suggest that the chemical compatibility of chimeric poliovirus-mediated immunomodulatory signaling is robust.

Example 2. Minimum cellular contact time required for chimeric poliovirus lerapolturev infectivity

The purpose of this study was to evaluate the minimum period of time lerapolturev needs to be in contact with cells in order to achieve substantial cellular infectivity. U87 glioma epithelial-like cells (NCI reference standard Lot# L1310001) were seeded at 10,000 cells per well and allowed to settle for 16 hours prior to the start of treatment. Following this, cells were incubated with lerapolturev for 5 min, 15 min, 30 min, 1 hour, 2 hours, 44 hours (positive control) or with mock sample (0 min, negative control) (FIG. 3A). Virus dilutions were prepared at MOI 20, 6.6, and 2. At each timepoint, media containing virus was aspirated, then cells were washed with fresh media (without virus) 3 times. Following this, growth media was added to the plate for up to 44 hours (including the various incubation times with lerapolturev). On the last day of the experiment, MTS reagent was incubated with cells for 4 hours then SDS was added and absorbance measurements at 490 nm were collected using a Spectramax ID5 multi-mode microplate reader.

All time points under 30 minutes of lerapolturev contact led to little infectivity. The minimum amount of lerapolturev cellular contact time observed that retained lerapolturev infectivity was 30 min or longer. The 30 min, 1 hour, and 2 hour time points all retained substantial lerapolturev infectivity that was comparable with the positive control condition at all MOI conditions (FIG. 3B).

Next, A375 melanoma cells (ATCC, CR-1619) were treated with lerapolturev at different MOI (2, 6.6, 20) for 5 min, 15 min, 30 min, 60 min, 2 hr, or 24 hr. After reaching the specific incubation time, growth media containing virus were removed, cells were washed and incubated with virus free growth media for 48 hr total (including the various incubation times with lerapolturev). At 48 hours, supernatant samples were collected, and IFN-β secretion was analyzed using the human IFN-β Quantikine ELISA Kit (R&D Systems, DIFNB0).

No significant IFN-β release (lerapolturev immunomodulation activity) was observed with contact time under 30 min, with 5 min and 15 min contact times showing little to no IFN-β signal (FIG. 3C). As contact time increased, however, increased IFN-β secretion was observed, with the

24 hr contact time reaching the highest IFN-β release when compared with all other time course groups. In comparison, the positive control sample (contact time = 48 hr) nearly doubled the measured the concentration of secreted IFN-β for the 24 hr contact time group at each MOI value. Within each contact time group, dose-dependent increases in IFN-β secretion were observed with increasing MOI values (2.0 to 6.6, 6.6 to 20).

These data suggest that incubating cells for between at about 30 minutes and at about 2 hours results in cellular lerapolturev infectivity and immunomodulatory signaling regulated by INF-β. These data further suggest that administering a chimeric poliovirus (e.g., lerapolturev) to a bladder by intravesical instillation for a time period of at about 2 hours is sufficient to induce robust bladder cell infectivity and an anti-tumor immunological response.

Example 3. Proposed lerapolturev intravesical instillation clinical trial

The purpose of this substudy is to evaluate different methods of administration of PVSRIPO into tumors located within the bladder mucosa. Two cohorts of patients will be enrolled in this portion of the study, including Cohort E and F. Both Cohort E and F will evaluate the administration of PVSRIPO monotherapy in patients with recurrent NMIBC intended for TURBT or cystectomy; patients enrolled in Cohort E will receive PVSRIPO via intravesical instillation while patients enrolled in Cohort F will receive PVSRIPO via intratumoral injection via cystoscopy.

Cohort E has been designed to evaluate 2 different dose levels of PVSRIPO using a 3+3 dose escalation approach (Figure 4). Three patients will initially be treated with a total dose of 2 $x10^9$ $TCID_{50}$ (Low Dose Cohort E) administered by intravesical instillation and the frequency of DLTs assessed. Each dose of PVSRIPO will be administered in a final volume of 30 ml by intravesical instillation. A urinary catheter will be inserted into the urethra under aseptic conditions according to local hospital protocol and the bladder drained completely. Using a catheter adapter with the syringe, the PVSRIPO suspension will then be instilled into the bladder via the catheter over a period of 3 to 5 minutes. After instillation the catheter will be removed and the patient instructed to retain the instilled suspension in the bladder for a period of 2 hours. During this period, care should be taken to ensure that the instilled suspension has sufficient contact with the whole mucosal surface of the bladder. The patient should be encouraged to mobilize or, if

lying down, to rotate between prone, supine, left lateral, and right lateral positions every 15 minutes.

After 2 hours the patient should void the instilled suspension directly into a toilet. After bladder evacuation, the next first voided urine sample should be collected for PVSRIPO Shedding. The patient should be advised to limit their fluid intake for 4 hours prior to instillation and until bladder evacuation is permitted (i.e. 2 hours after instillation).

The decision to escalate/de-escalate the PVSRIPO dose will be based on the presence of DLTs observed during the 14 days following administration of PVSRIPO in consultation with the independent Data Safety Monitoring Committee (DSMC).

Provided that none of the initial 3 patients experience a DLT, the dose will be considered not to have exceeded the maximally tolerated dose (MTD) and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (High Dose Cohort E) via intravesical instillation. However, if 1 out of 3 patients enrolled in the Low Dose Cohort E experience a DLT, the cohort will be expanded by enrolling 3 additional patients. If no additional patient experiences a DLT, then the dose will be considered not to have exceeded the MTD and the next cohort of patients (n=3) will receive a total dose of $1x10^{10}$ $TCID_{50}$ (High Dose Cohort E) via intravesical instillation. In contrast, if a 2nd patient in the Low Dose Cohort E experiences a DLT during the initial enrollment of 3 patients--or expanded enrollment to 6 patients--then the maximum dose will have been exceeded, and the dose will be de-escalated to a dose of $2x10^{8}$ $TCID_{50}$ (Lowest Dose Cohort E). The evaluation of either the High Dose or Lowest Dose Cohort E will follow the same process as outlined above and in Figure 1.

Once enrollment in Cohort E has been completed, Cohort F will open to evaluate intravesical instillation of PVSRIPO after a sequence of a 100 ml saline wash, 75 ml 5% DDM wash, and 100 ml saline wash in patients (n=3) with recurrent NMIBC using the highest dose evaluated in Cohort E that does not exceed the maximum tolerated dose. The purpose of Cohort F is to assess if PVSRIPO infection of cells within the bladder mucosa is facilitated by pretreatment with a detergent able to disrupt the GAG layer of the epithelium.

In the event tissue from a TURBT or cystectomy in a given patient is not available after completion of standard of care pathologic review, that patient may be replaced as long as the dose administered to the replacement patient does not exceed the maximum tolerated dose.

Example 4. Exemplary intravesical instillation administration schedules of chimeric poliovirus

A chimeric poliovirus is to be administered by intravesical instillation in several different potential administration schedules. For example, exemplary administration schedules of a chimeric poliovirus (e.g., lerapolturev) are illustrated in FIG. 5A – D. In one administration schedule, a chimeric poliovirus (e.g., lerapolturev) is administered by intravesical instillation on the first day of each week of a 6-week induction cycle (FIG. 5A). In another administration schedule, a chimeric poliovirus (e.g., lerapolturev) is administered by intravesical instillation on the first day of each week of two consecutive 6-week induction cycles (FIG. 5B). In another administration schedule, a chimeric poliovirus (e.g., lerapolturev) is administered by intravesical instillation on the first day of each week of a 6-week induction cycle which comprises an induction phase followed by intravesical instillation administration of a chimeric poliovirus (e.g., lerapolturev) on the first day of the first three weeks of months 3, 6, 12, 18, 24, 30, and 36 months following the beginning of the induction cycle, which comprises a maintenance phase (FIG. 5C). The one or more maintenance phases comprise three weekly intravesical instillation administrations of a chimeric poliovirus in maintenance cycles occurring at months 1, 3, 9, 15, 21, 27, and 33 of the maintenance phase.

In another administration schedule, a chimeric poliovirus (e.g., lerapolturev) is administered by intravesical instillation on the first day of each week of a 6-week induction cycle which comprises an induction phase followed by intravesical instillation administration of a chimeric poliovirus (e.g., lerapolturev) on the first day of the first week of months 3, 6, 12, 18, 24, 30, and 36 months following the beginning of the induction cycle, which comprises a maintenance phase (FIG. 5C). The one or more maintenance phases comprise one intravesical instillation administration of a chimeric poliovirus in maintenance cycles occurring at months 1, 3, 9, 15, 21, 27, and 33 of the maintenance phase.

In some embodiments, prior to each lerapolturev administration, the patient is administered a series of pre-washes comprising saline and 5% DDM. In some embodiments, the intravesical instillation of PVSRIPO occurs after a sequence of a 100 ml saline wash, a 75 ml 5% DDM wash, and a 100 ml saline wash.

CLAIMS

What is Claimed:

1. A method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, wherein the induction phase comprises one or more 6-week treatment cycles comprising administering to the subject an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week of the treatment cycle.
2. A method of treating a human subject having bladder cancer, wherein the treatment comprises an induction phase, the induction phase comprising two 6-week treatment cycles, wherein each 6-week treatment cycle comprises administering to the subject an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week.
3. A method of treating a human subject having bladder cancer, wherein the treatment comprises a maintenance phase, wherein the maintenance phase comprises one or more treatment cycles, wherein each treatment cycle comprises the administration of an effective amount of a chimeric poliovirus once a week by intravesical instillation, wherein each treatment cycle comprises 1-week, 2-weeks, 3-weeks, 4-weeks, or 6 weeks, and wherein the initiation of each treatment cycle is 4 weeks apart, 6 weeks apart, 8 weeks apart, 10 weeks apart, 3 months apart, 6 months apart, or a combination thereof.
4. The method of claim 3, wherein the treatment cycle is 1-week, 2-weeks, or 3-weeks.
5. The method of claim 3, wherein the initiation of each of the one or more treatment cycles are 3-months apart or 6-months apart.
6. The method of claim 3, wherein the human subject is administered a maintenance phase only.
7. The method of any of claims 1 or 2, wherein the maintenance phase of claim 3 is administered following the cessation of the induction phase.
8. The method of claim 7, wherein the maintenance phase is administered at least one or more weeks following cessation of the induction phase.
9. The method of claim 7, wherein the treatment comprises:

a) an induction phase comprising a 6-week treatment cycle, wherein the 6-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week of the treatment cycle; and,

b) a maintenance phase comprising 3-week treatment cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, wherein each 3-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week of the 3-week treatment cycle.

10. The method of claim 7, wherein the treatment comprises:

a) an induction phase comprising a 6-week treatment cycle, wherein the 6-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week; and,

b) a maintenance phase comprising 1-week treatment cycles starting at the beginning of months 3, 6, 12, 18, 24, 30, and 36 following the start of the induction phase, wherein each 1-week treatment cycle comprises administering to the patient an effective amount of a chimeric poliovirus by intravesical instillation on the first day of each week.

11. The method of any of claims 1-10, wherein the chimeric poliovirus is first administered during the treatment cycle within 7 days of transurethral resection of the bladder tumor (TURBT).

12. The method of any of claims 1-10, wherein the chimeric poliovirus is first administered during the treatment cycle within 24 hours of transurethral resection of the bladder tumor (TURBT).

13. A method of treating a human subject having bladder cancer, wherein the treatment comprises a single administration of an effective amount of a chimeric poliovirus intravesical instillation, wherein the chimeric poliovirus is administered within 24 hours of transurethral resection of the bladder tumor (TURBT).

14. The method of any of claims 1-13, wherein the chimeric poliovirus comprises a Sabin type I strain of poliovirus with a human rhinovirus 2 (HRV2) internal ribosome entry site (IRES)

in the poliovirus 5' untranslated region between the poliovirus cloverleaf and the poliovirus open reading frame.

15. The method of claim 14, wherein the chimeric poliovirus comprises lerapolturev.

16. The method of any of claims 1-15, wherein the bladder cancer is selected from a non-muscle invasive bladder cancer (NMIBC) or a muscle invasive bladder cancer (MIBC).

17. The method of claim 16, wherein the bladder cancer is a non-muscle invasive bladder cancer (NMIBC).

18. The method of claim 17, wherein the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient is ineligible for cystectomy.

19. The method of claim 17, wherein the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) with papillary tumors in a patient who has elected not to undergo cystectomy.

20. The method of claim 17, wherein the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient is ineligible for cystectomy.

21. The method of claim 17, wherein the NMIBC is a Bacillus-Calmette-Guerin (BCG)-unresponsive, high-risk NMIBC with carcinoma in situ (CIS) without papillary tumors in a patient who has elected not to undergo cystectomy.

22. The method of claim 17, wherein the NMIBC is a carcinoma in situ (CIS) of the urinary bladder.

23. The method of claim 17, wherein the NMIBC is a primary or recurrent stage Ta and/or T1 papillary bladder cancer tumors following transurethral resection of bladder tumor(s) (TURBT).

24. The method of any of claims 1-15, wherein the human patient is an adult with a high risk of disease recurrence and/or progression.

25. The method of claim 16, wherein the bladder cancer is muscle invasive bladder cancer (MIBC).

26. The method of claim 25, wherein the MIBC is a resectable cisplatin-ineligible/refusal MIBC.

27. The method of claim 25, wherein the MIBC is a locally advanced or metastatic bladder cancer that has not progressed with first-line platinum-containing chemotherapy.
28. The method of any of claims 1-27, wherein the subject previously received cancer therapy.
29. The method of claim 28, wherein the previously received cancer therapy is selected from exposure to an intravesical agent, a radiation therapy, a chemotherapeutic agent, an immune checkpoint inhibitor (ICI), or a combination thereof.
30. The method of claim 29, wherein the ICI is selected from a PD-1 inhibitor or a PD-L1 inhibitor.
31. The method of claim 30, wherein the ICI comprises a PD-1 inhibitor.
32. The method of claim 31, wherein the PD-1 inhibitor is selected from pembrolizumab or nivolumab.
33. The method of claim 31, wherein the PD-1 inhibitor is pembrolizumab.
34. The method of claim 31, wherein the PD-1 inhibitor is nivolumab.
35. The method of claim 30, wherein the ICI comprises a PD-L1 inhibitor.
36. The method of claim 35, wherein the PD-L1 inhibitor is selected from atezolizumab, durvalumab, or avelumab.
37. The method of claim 35, wherein the PD-L1 inhibitor is atezolizumab.
38. The method of claim 5, wherein the PD-L1 inhibitor is durvalumab.
39. The method of claim 35, wherein the PD-L1 inhibitor is avelumab.
40. The method of any of claims 1-39, wherein the chimeric poliovirus is administered at a dose of between about $1.0x10^8$ to about $5.0.x10^{10}$ $TCID_{50}$.
41. The method of any of claims 1-39, wherein the chimeric poliovirus is administered at a dose of about $2.0x10^8$ $TCID_{50}$.
42. The method of any of claims 1-39, wherein the chimeric poliovirus is administered at a dose of about $2.0x10^9$ $TCID_{50}$.
43. The method of any of claims 1-39, wherein the chimeric poliovirus is administered at a dose of about $1.0x10^{10}$ $TCID_{50}$.
44. The method of any of claims 1-39, wherein the chimeric poliovirus is administered in a volume of about 30 mL.

45. The method of any of claims 1-44, further comprising administering a detergent via intravesical instillation prior to or at the same time as administering the chimeric poliovirus.

46. The method of claim 45, wherein the detergent is selected from DDM, Tween-80, or SIM3.

47. The method of claim 46, wherein the detergent is N-dodecyl-β-D-maltoside (DDM).

48. The method of claim 46, wherein the detergent is Tween-80.

49. The method of claim 46, wherein the detergent is SIM3.

50. The method of any of claims 1-49, wherein the method further comprises administering the chimeric poliovirus in combination with an additional bladder cancer therapy selected from a chemotherapy, a radiation, an intravesical agent, an immune checkpoint inhibitor (ICI), or a combination thereof.

51. The method of claim 50, wherein the additional bladder cancer comprises an immune checkpoint inhibitor (ICI).

52. The method of claim 51, wherein the ICI is selected from a programmed cell death -1 (PD-1) inhibitor, a programmed cell death-ligand 1 (PD-L1) inhibitor, a cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitor, a lymphocyte-activation gene 3 (LAG-3) inhibitor, a T-cell immunoglobulin mucin-3 (TIM-3) inhibitor, a T cell immunoreceptor with Ig and ITIM domains (TIGIT) inhibitor, a V-domain Ig suppressor of T-cell activation (VISTA) inhibitor, a carcinoembryonic antigen cell adhesion molecule (CEACAM) inhibitor, a sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) inhibitor, a CD47 inhibitor, a CD39 inhibitor, or a B and T lymphocyte attenuator (BTLA) protein inhibitor, a B7-H3/CD276 inhibitor, an indoleamine 2,3-dioxygenase (IDO) inhibitor, a killer immunoglobin-like receptor (KIR) inhibitor, or a combination thereof.

53. The method of claim 52, wherein the ICI is a PD-1 inhibitor.

54. The method of claim 53, wherein the PD-1 inhibitor is selected from nivolumab, pembrolizumab, pidilizumab, AMP-224, sasanlimab, spartalizumab, cemiplimab, retifanlimab, tislelizumab, camrelizumab, CS1003, or dostarlimab.

55. The method of claim 54, wherein the PD-1 inhibitor is pembrolizumab.

56. The method of claim 54, wherein the PD-1 inhibitor is nivolumab.

57. The method of claim 52, wherein the ICI is a PD-L1 inhibitor.

58. The method of claim 57, wherein the PD-L1 inhibitor is selected from atezolizumab, durvalumab, avelumab, envafolimab, BMS-936559, lodapolimab, cosibelimab, sugemalimab, adebrelimab, CBT-502, or BGB-A333.

59. The method of claim 58, wherein the PD-L1 inhibitor is atezolizumab.

60. The method of claim 52, wherein the ICI is a CTLA-4 inhibitor.

61. The method of claim 60, wherein the CTLA-4 inhibitor is selected from the group consisting of ipilimumab and tremelimumab.

62. The method of claim 52, wherein the ICI is a LAG-3 inhibitor.

63. The method of claim 62, wherein the LAG-3 inhibitor is selected from relatlimab, GSK2831781, eftilagimod alpha, leramilimab, MK-4280, REGN3767, TSR-033, BI754111, Sym022, tebotelimab, FS118, LAG-526, favezelimab, CB213, SNA-03, INCAGN02385, RO7247669, IBI323, EMB-02, or AVA-0017.

64. The method of claim 52, wherein the ICI is a TIM-3 inhibitor.

65. The method of claim 64, wherein the TIM-3 inhibitor is selected from TSR-022, MBG453, Sym023, INCAGN2390, LY3321367, BMS-986258, SHR-1702, RO7121661, sabatolimab, cobolimab, RG7769, MAS-825, BGBA425, AZD7789, TQB2618, or NB002.

66. The method of claim 52, wherein the ICI is a TIGIT inhibitor.

67. The method of claim 66, wherein the TIGIT inhibitor is selected from MK-7684, etigilimab/OMP-313 M32, tiragolumab/MTIG7192A/RG-6058, BMS-986207, AB-154, ASP-8374, Vibostolimab, AZD2936, ASP8374, Domvanalimab, IBI939, Ociperlimab, EOS884448, SEA-TGT, COM902, MPH-313, M6223, HLX53, JS006, mAb-7, SHR-1708, BAT6005, GS02, RXI-804, NB6253, ENUM009, CASC-674, AJUD008, or AGEN1777.

68. The method of claim 52, wherein the ICI is a VISTA inhibitor.

69. The method of claim 52, wherein the ICI is a CEACAM inhibitor.

70. The method of claim 52, wherein the ICI is a Siglec-15 inhibitor.

71. The method of claim 52, wherein the ICI is a CD47 inhibitor.

72. The method of claim 52, wherein the ICI is a CD39 inhibitor.

73. The method of claim 52, wherein the ICI is a BTLA protein inhibitor.

74. The method of claim 52, wherein the ICI is a B7-H3/CD276 inhibitor.

75. The method of claim 52, wherein the ICI is an indoleamine 2,3-dioxygenase (IDO) inhibitor.

76. The method of claim 52, wherein the ICI is a killer immunoglobin-like receptor (KIR) inhibitor.

77. The method of any of claims 50-76, wherein the ICI is administered at the standard recommended dose and schedule as described on an FDA approved label.

78. The method of claim 50, wherein the additional bladder cancer therapy comprises a chemotherapy.

79. The method of claim 78, wherein the chemotherapy is selected from cisplatin, carboplatin, oxaliplatin, gemcitabine, mitomycin C, doxorubicin, epirubicin, docetaxel, methotrexate, vinblastine, cabazitaxel, or a combination thereof.

80. The method of claim 50, wherein the additional bladder cancer therapy comprises an intravesical agent.

81. The method of claim 80, wherein the intravesical agent is BCG.

82. The method of claim 81, wherein the immunomodulatory intravesical agent comprises BCG.

83. A pharmaceutical composition comprising a chimeric poliovirus and a pharmaceutically acceptable carrier, formulated for intravesical instillation administration.

84. The pharmaceutical composition of claim 83, wherein the chimeric poliovirus comprises a Sabin type I strain of poliovirus with a human rhinovirus 2 (HRV2) internal ribosome entry site (IRES) in the poliovirus 5' untranslated region between the poliovirus cloverleaf and the poliovirus open reading frame.

85. The pharmaceutical composition of claim 83, wherein the chimeric poliovirus comprises lerapolturev.

86. The pharmaceutical composition of any of claims 83-85, wherein the chimeric poliovirus concentration is between about $2.0x10^8$ $TCID_{50}$ and about $5.0x10^{10}$ $TCID_{50}$.

87. The pharmaceutical composition of claim 86, wherein the chimeric poliovirus concentration is about $2.0x10^8$ $TCID_{50}$.

88. The pharmaceutical composition of claim 86, wherein the chimeric poliovirus concentration is about $2.0x10^9$ $TCID_{50}$.

89. The pharmaceutical composition of claim 86, wherein the chimeric poliovirus concentration is about $1.0x10^{10}$ $TCID_{50}$.

90. The method of claims 1-82, wherein prior to the intravesical instillation of the chimeric poliovirus, the subject is administered one or more pre-washes.

91. The method of claim 90, wherein the pre-wash comprises the intravesical administration of DDM.

92. The method of claim 91, wherein the DDM is administered in a concentration of from about 0.5% to about 10%.

93. The method of claim 92, wherein the DDM is administered in a concentration of from about 3% to about 6%.

94. The method of claim 93, wherein the DDM is administered in a concentration of about 5%.

95. The method of claims 91-94, wherein the DDM is administered in a volume of from about 50 mls to about 100 mls.

96. The method of claim 95, wherein the DDM is administered in a volume of about 75 mls.

97. The method of claims 91-96, wherein the DDM is administered and retained in the bladder from about 5 minutes to about 20 minutes.

98. The method of claim 97, wherein the DDM is administered and retained in the bladder for about 15 minutes ± about 5 minutes.

99. The method of claim 90, wherein the subject is administered a pre-wash which causes the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the bladder epithelium.

100. The method of claim 99, wherein the subject is administered a pre-wash comprising a surfactant.

101. The method of claim 100, wherein the surfactant is selected from Tween-80, sodium dodecyl sulfate, or cetylpyridinium chloride.

102. The method of claim 99, wherein the pre-wash comprises a calcium ion chelator.

103. The method of claim 102, wherein the calcium ion chelator is polycarbophil.

104. The method of claims 90-103, wherein the pre-wash comprises a saline wash.

105. The method of claim 104, wherein the saline pre-wash is administered prior to administration of a pre-wash which causes the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the bladder epithelium.

106. The method of claim 104, wherein the saline pre-wash is administered after administration of a pre-wash which causes the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the bladder epithelium.

107. The method of claim 104, wherein the saline pre-wash is administered both before and after administration of a pre-wash which causes the disruption of the polyanionic glycosaminoglycan (GAG) layer overlaying the bladder epithelium.

108. The method of claims 104-107, wherein the saline wash is administered and retained within the bladder for from about 2 minutes to about 10 minutes.

109. The method of claim 108, wherein the saline wash is administered and retained within the bladder for about 5 minutes.

110. The method of claims 104-109, wherein the saline wash is administered in a volume from about 50 mls to about 150 mls.

111. The method of claim 110, wherein the saline wash is administered in a volume of about 100 mls.

112. The method of claim 90, wherein the subject is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, iii) a third wash comprising about 100 ml saline.

113. The method of 112, wherein each saline wash is administered and retained in the bladder for about 5 minutes and the DDM wash is administered and retained in the bladder for about 15 minutes ± about 5 minutes.

114. A method of treating a human subject having non-muscle invasive bladder cancer comprising administering to the subject by intravesical instillation lerapolturev in a concentration between about $2.0x10^8$ $TCID_{50}$ and about $5.0x10^{10}$ $TCID_{50}$, wherein prior to the administration of lerapolturev, the subject is administered a series of pre-washes comprising i) a first wash comprising saline, ii) a second wash comprising DDM, iii) and a third wash comprising saline.

115. The method of claim 114, wherein the lerapolturev concentration is about $2.0x10^8$ $TCID_{50}$.

116. The method of claim 114, wherein the lerapolturev concentration is about $2.0x10^9$ $TCID_{50}$.

117. The method of claim 114, wherein the lerapolturev concentration is about $1.0x10^{10}$ $TCID_{50}$.

118. The method of claims 114-117, wherein the saline wash is administered and retained within the bladder for from about 2 minutes to about 10 minutes.

119. The method of claim 118, wherein the saline wash is administered and retained within the bladder for about 5 minutes.

120. The method of claims 114-119, wherein the saline wash is administered in a volume from about 50 mls to about 150 mls.

121. The method of claim 120, wherein the saline wash is administered in a volume of about 100 mls.

122. The method of claims 114-121, wherein the DDM is administered in a concentration of from about 0.5% to about 10%.

123. The method of claim 122, wherein the DDM is administered in a concentration of from about 3% to about 6%.

124. The method of claim 123, wherein the DDM is administered in a concentration of about 5%.

125. The method of claims 114-124, wherein the DDM is administered in a volume of from about 50 mls to about 100 mls.

126. The method of claim 125, wherein the DDM is administered in a volume of about 75 mls.

127. The method of claims 114-126, wherein the DDM is administered and retained in the bladder from about 5 minutes to about 20 minutes.

128. The method of claim 127, wherein the DDM is administered and retained in the bladder for about 15 minutes ± about 5 minutes.

129. The method of claim 114, wherein the subject is administered a pre-wash sequence in the order comprising i) a first wash comprising about 100 ml saline, ii) a second wash comprising about 75 ml 5% DDM, iii) a third wash comprising about 100 ml saline.

130. The method of 129, wherein each saline wash is administered and retained in the bladder for about 5 minutes and the DDM wash is administered and retained in the bladder for about 15 minutes ± about 5 minutes.

131. The method of claims 1-14, wherein the chimeric poliovirus is administered in a pharmaceutical composition selected from the pharmaceutical composition of claims 83-89.

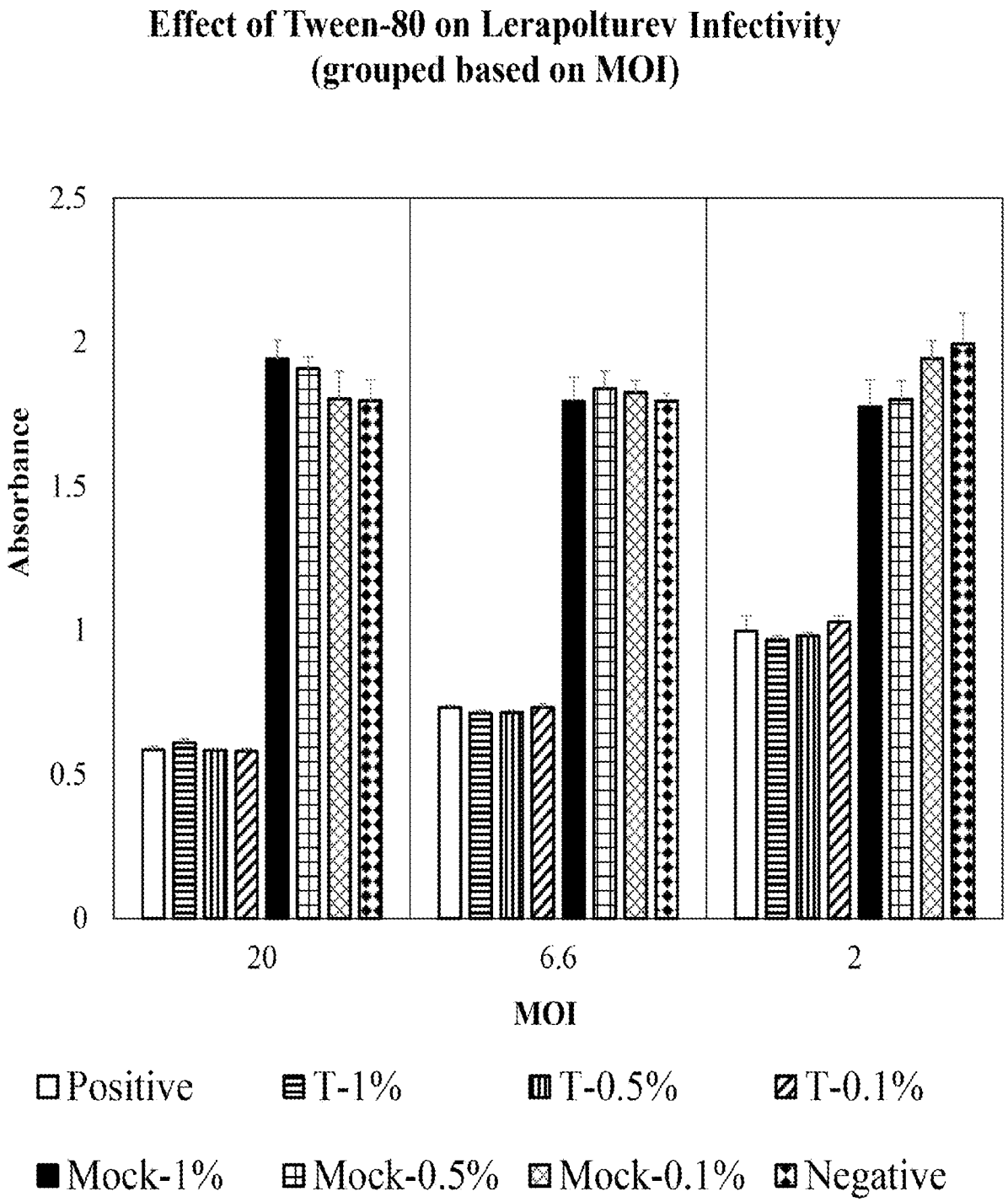
Effect of Tween-80 on Lerapolturev Infectivity
(grouped based on MOI)
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive
T-1%
T-0.5%
T-0.1%
Mock-1%
Mock-0.5%
Mock-0.1%
Negative

FIG. 1A

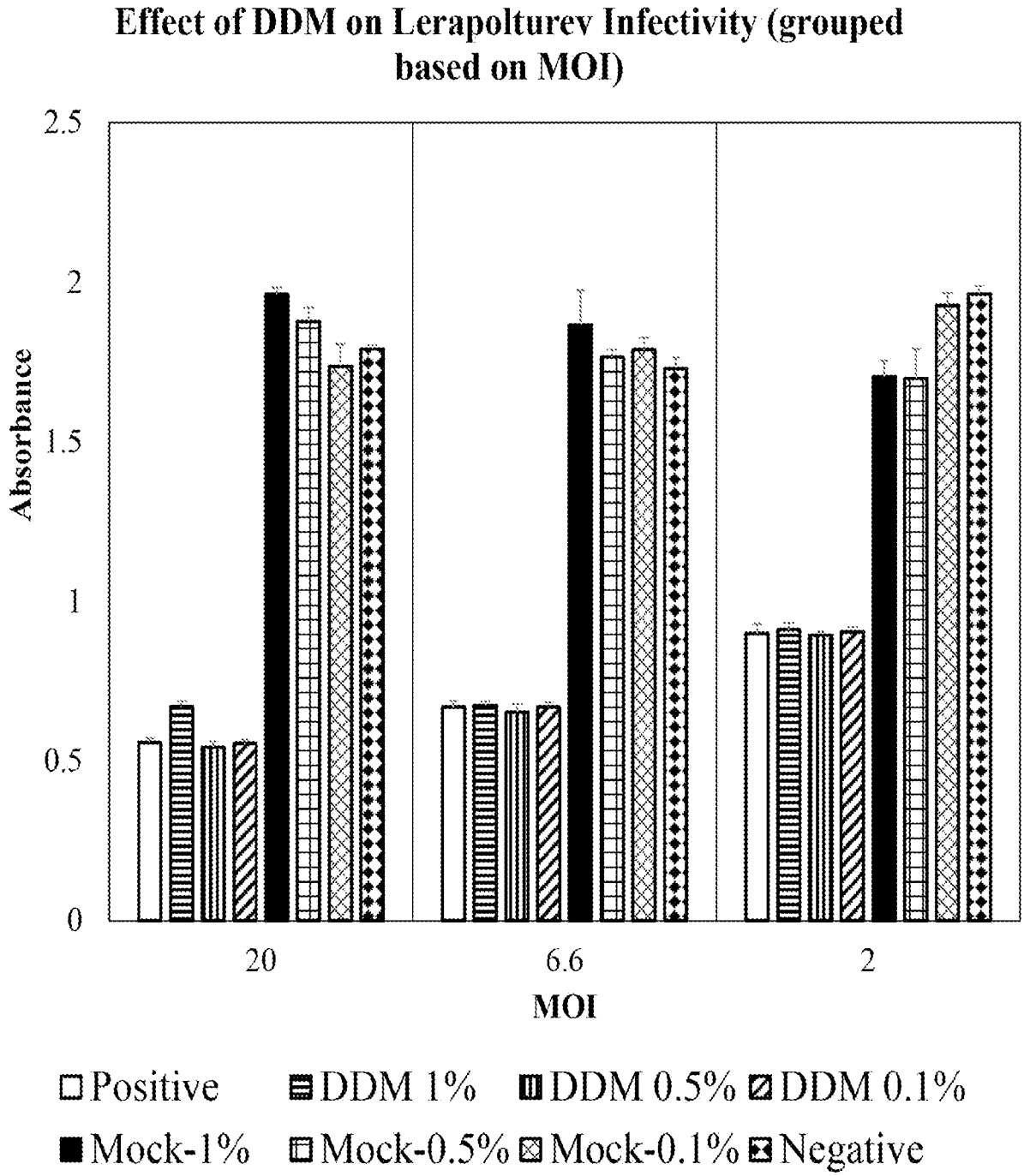
Effect of DDM on Lerapolturev Infectivity (grouped based on MOI)
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive
DDM 1%
DDM 0.5%
DDM 0.1%
Mock-1%
Mock-0.5%
Mock-0.1%
Negative

FIG. 1B

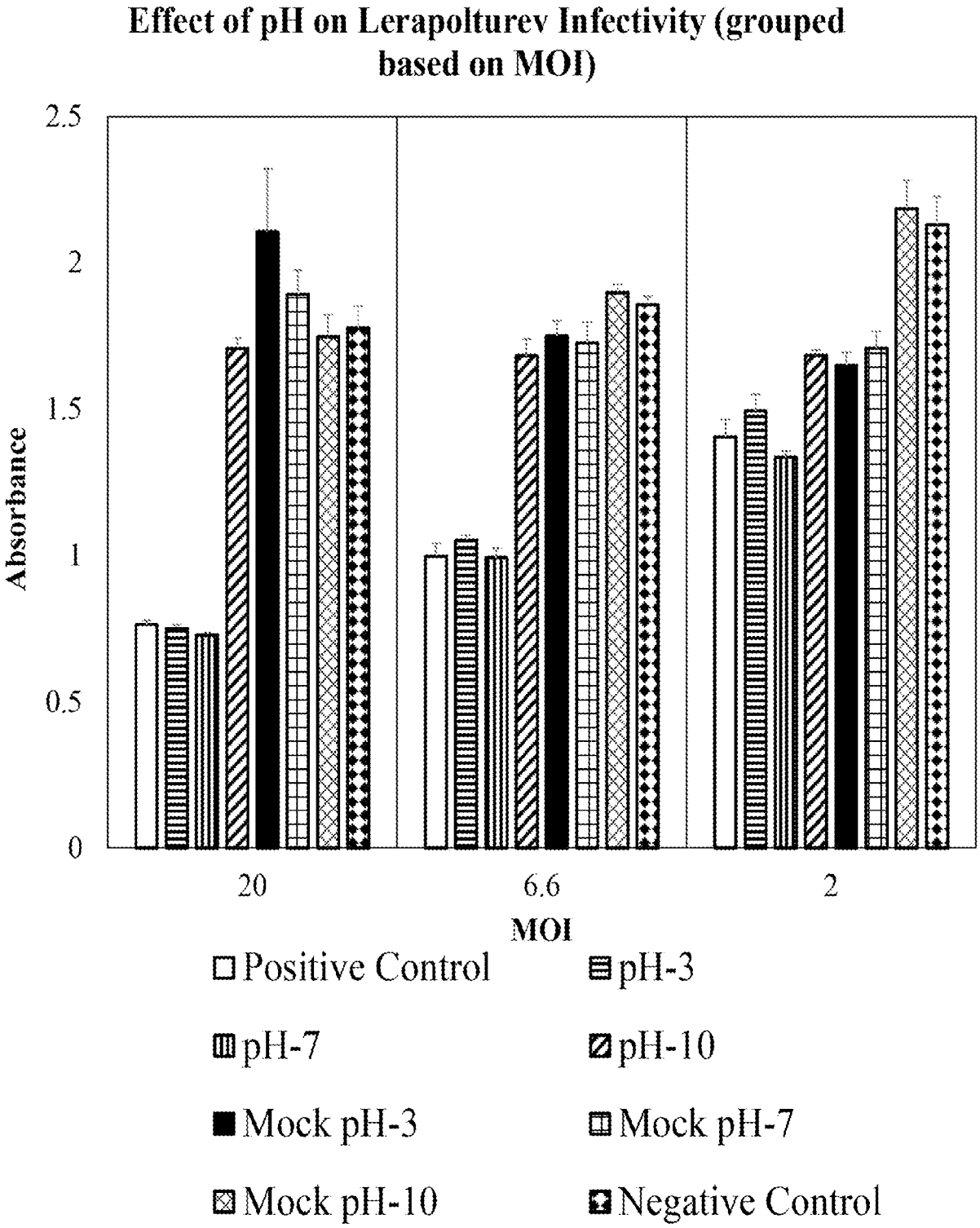
Effect of pH on Lerapolturev Infectivity (grouped based on MOI)
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive Control
pH-3
pH-7
pH-10
Mock pH-3
Mock pH-7
Mock pH-10
Negative Control

FIG. 1C

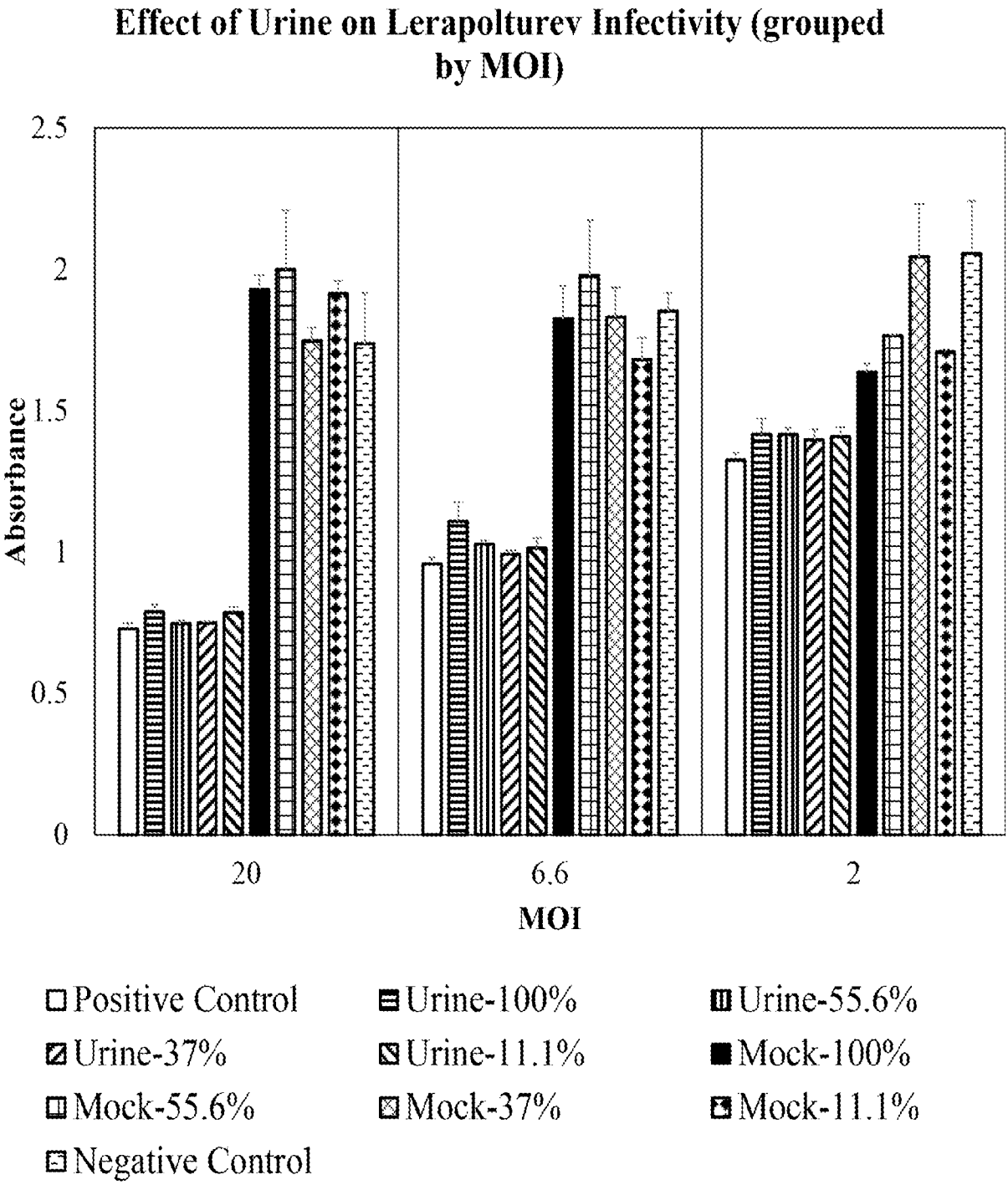
Effect of Urine on Lerapolturev Infectivity (grouped by MOI)
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive Control
Urine-100%
Urine-55.6%
Urine-37%
Urine-11.1%
Mock-100%
Mock-55.6%
Mock-37%
Mock-11.1%
Negative Control

FIG. 1D

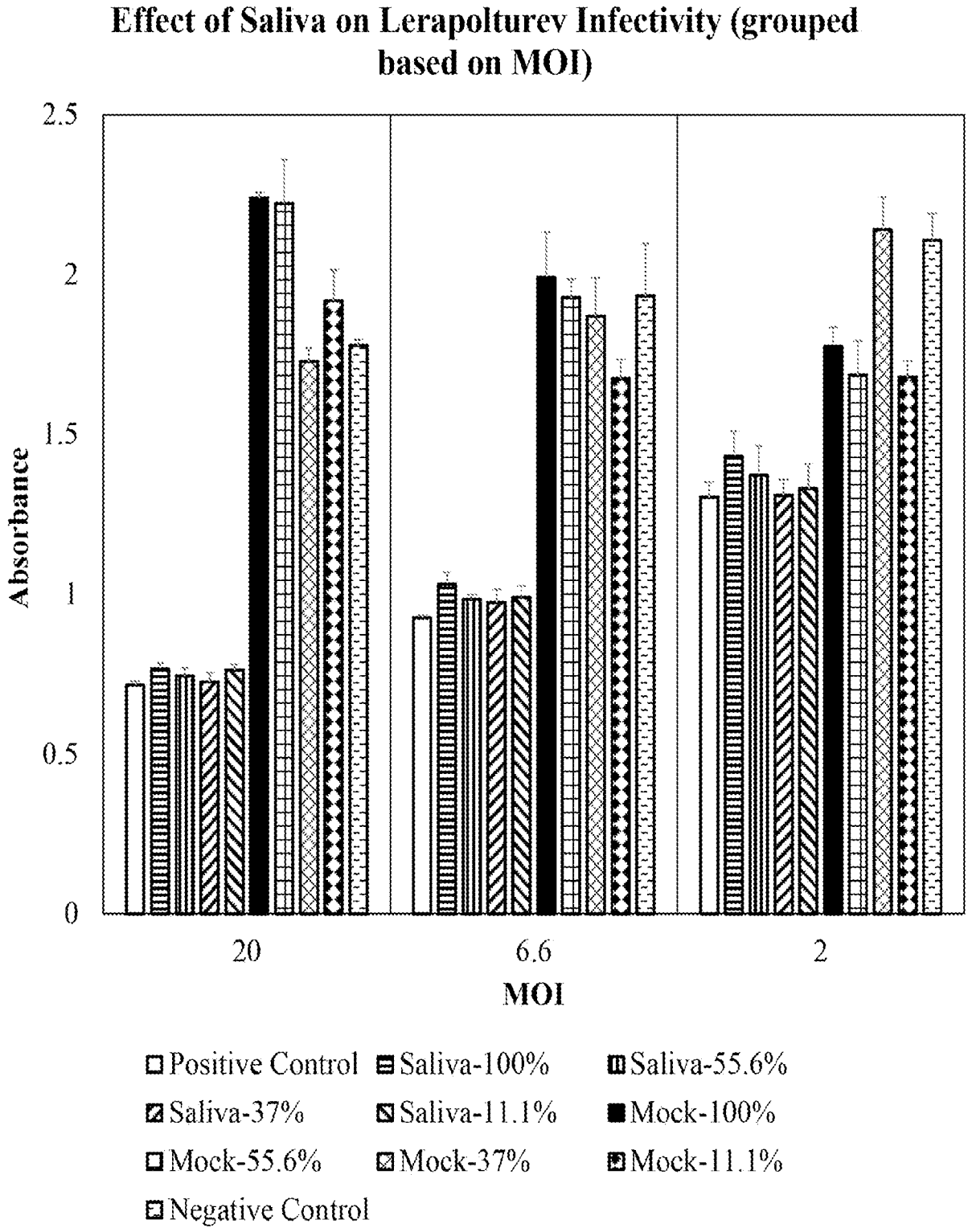
Effect of Saliva on Lerapolturev Infectivity (grouped based on MOI)
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive Control
Saliva-100%
Saliva-55.6%
Saliva-37%
Saliva-11.1%
Mock-100%
Mock-55.6%
Mock-37%
Mock-11.1%
Negative Control

FIG. 1E

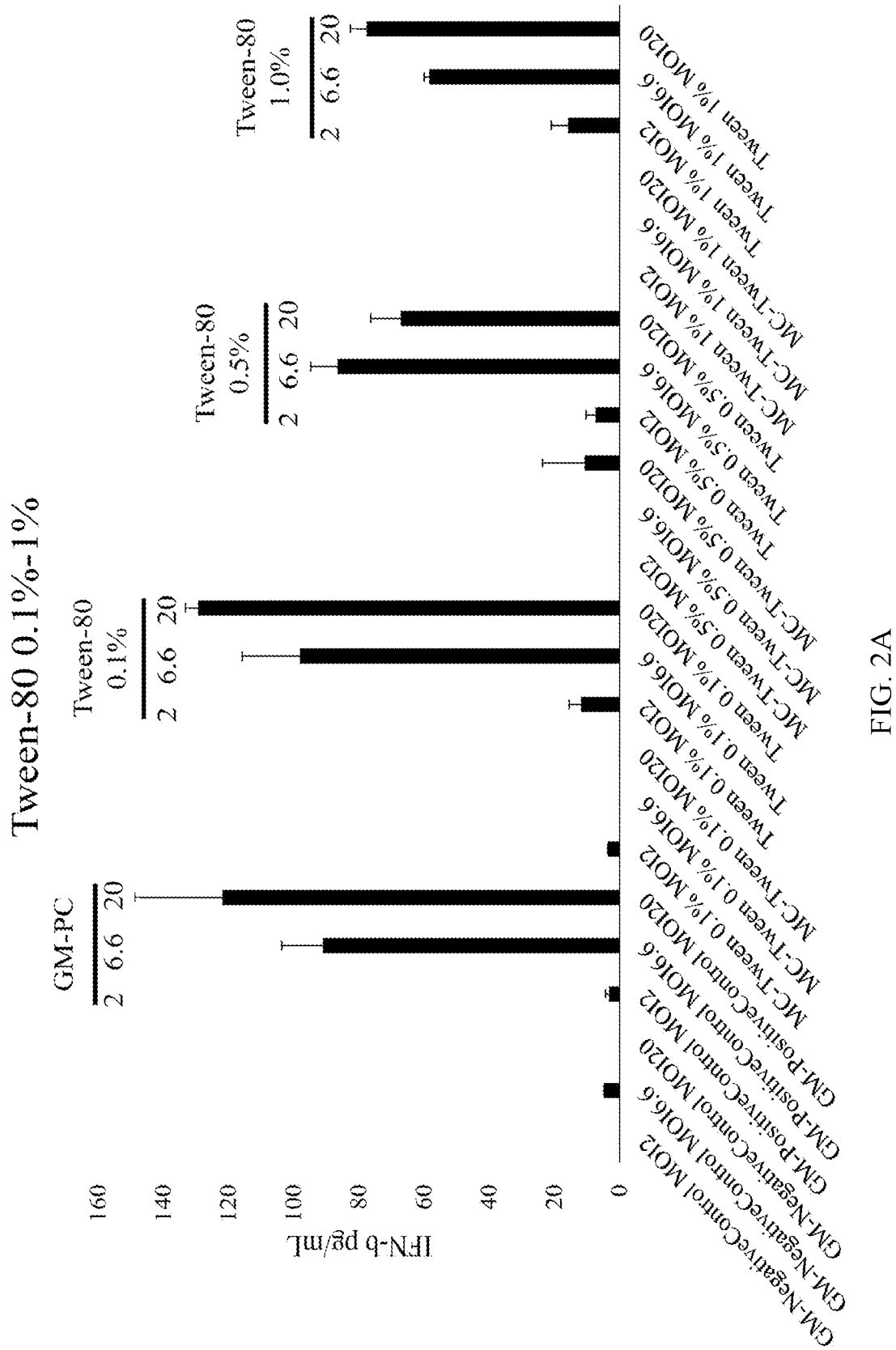
Tween-80 0.1%-1%
GM-PC
2 6.6 20
Tween-80
0.1%
2 6.6 20
Tween-80
0.5%
2 6.6 20
Tween-80
1.0%
2 6.6 20
IFN-b pg/mL
0
20
40
60
80
100
120
140
160
GM-NegativeControl MOI2
GM-NegativeControl MOI6.6
GM-NegativeControl MOI20
GM-PositiveControl MOI2
GM-PositiveControl MOI6.6
GM-PositiveControl MOI20
MC-Tween 0.1% MOI2
MC-Tween 0.1% MOI6.6
MC-Tween 0.1% MOI20
MC-Tween 0.5% MOI2
MC-Tween 0.5% MOI6.6
MC-Tween 0.5% MOI20
Tween 0.1% MOI2
Tween 0.1% MOI6.6
Tween 0.1% MOI20
Tween 0.5% MOI2
Tween 0.5% MOI6.6
Tween 0.5% MOI20
MC-Tween 1% MOI2
MC-Tween 1% MOI6.6
MC-Tween 1% MOI20
Tween 1% MOI2
Tween 1% MOI6.6
Tween 1% MOI20

FIG. 2A

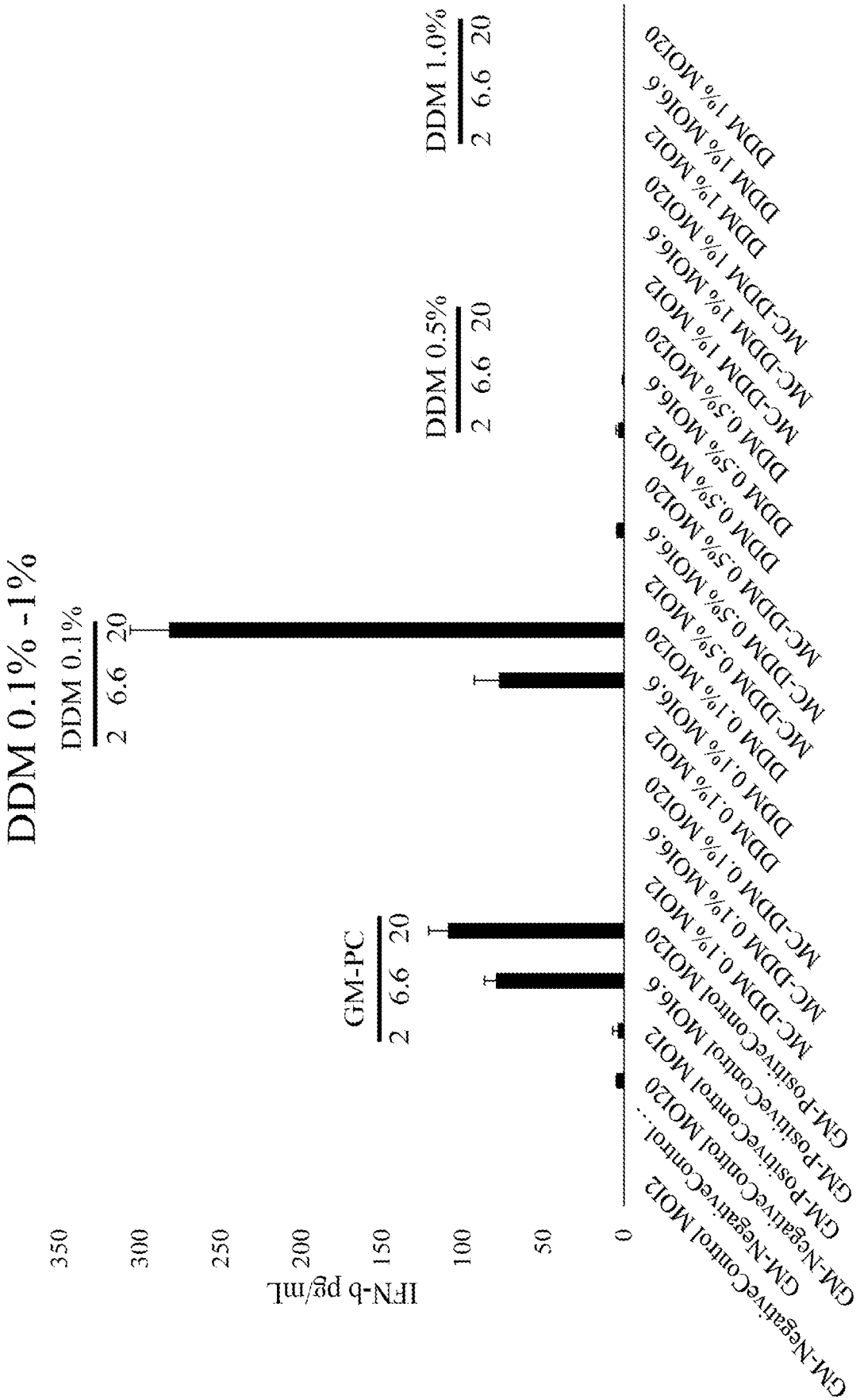
DDM 0.1% -1%
DDM 0.1%
2 6.6 20
DDM 0.5%
2 6.6 20
DDM 1.0%
2 6.6 20
GM-PC
2 6.6 20
IFN-b pg/mL
350
300
250
200
150
100
50
0
GM-Negativec ontrol MOI2
GM-Negativec ontrol...
GM-Negativec ontrol MOI20
GM-Positivec ontrol MOI2
GM-Positivec ontrol MOI6.6
GM-Positivec ontrol MOI20
MC-DDM 0.1% MOI2
DDM 0.1% MOI6.6
MC-DDM 0.1% MOI20
DDM 0.5% MOI2
MC-DDM 0.5% MOI6.6
DDM 0.5% MOI20
MC-DDM 1% MOI2
DDM 1% MOI6.6
MC-DDM 1% MOI20
DDM 1% MOI2
DDM 1% MOI6.6
DDM 1% MOI20

FIG. 2B

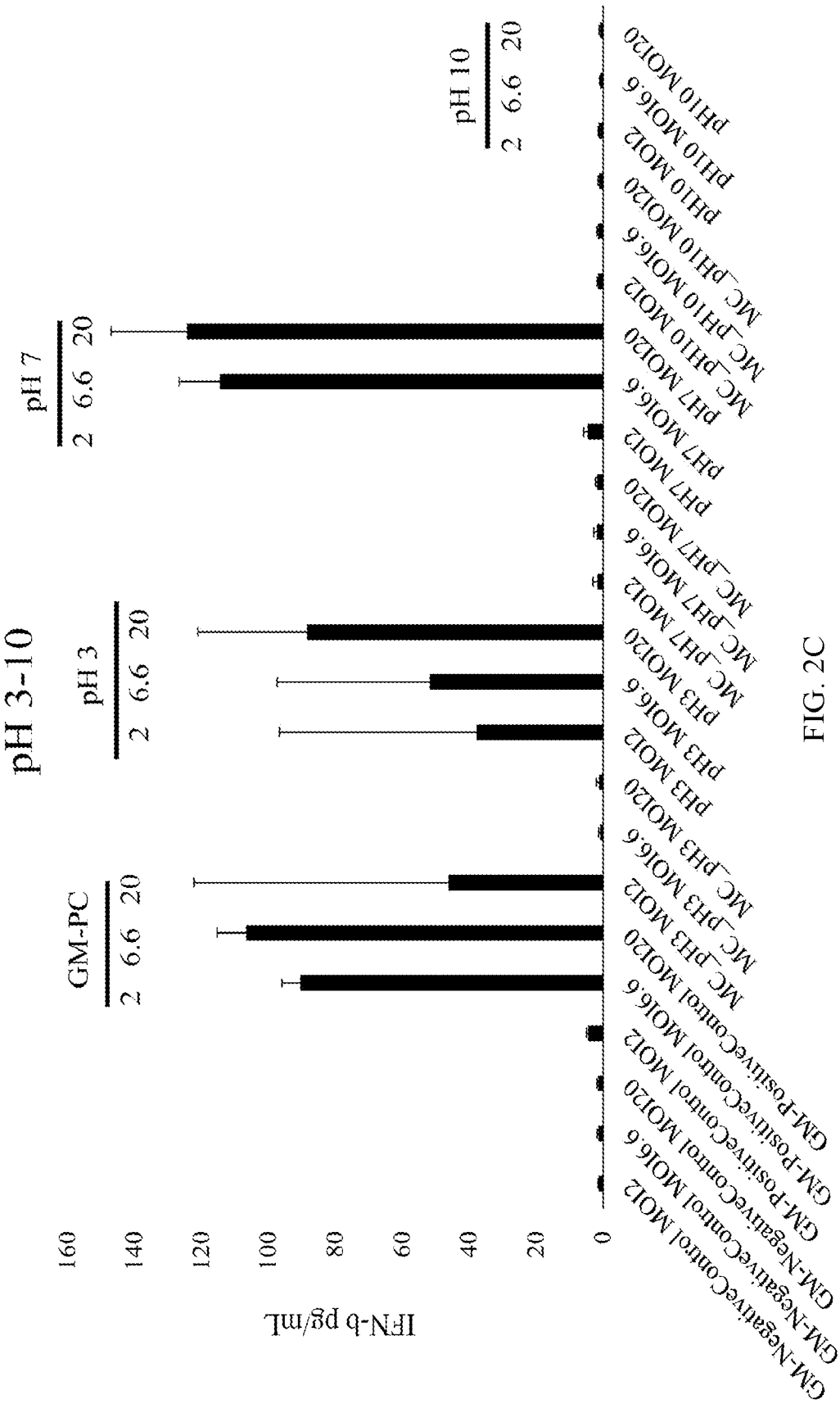
pH 3-10
GM-PC
2 6.6 20
pH 3
2 6.6 20
pH 7
2 6.6 20
pH 10
2 6.6 20
IFN-b pg/mL
0
20
40
60
80
100
120
140
160
GM-NegativeControl MOI2
GM-NegativeControl MOI6.6
GM-NegativeControl MOI20
GM-PositiveControl MOI2
GM-PositiveControl MOI6.6
GM-PositiveControl MOI20
MC_pH3 MOI2
MC_pH3 MOI6.6
MC_pH3 MOI20
pH3 MOI2
pH3 MOI6.6
pH3 MOI20
MC_pH7 MOI2
MC_pH7 MOI6.6
MC_pH7 MOI20
pH7 MOI2
pH7 MOI6.6
pH7 MOI20
MC_pH10 MOI2
MC_pH10 MOI6.6
MC_pH10 MOI20
pH10 MOI2
pH10 MOI6.6
pH10 MOI20

FIG. 2C

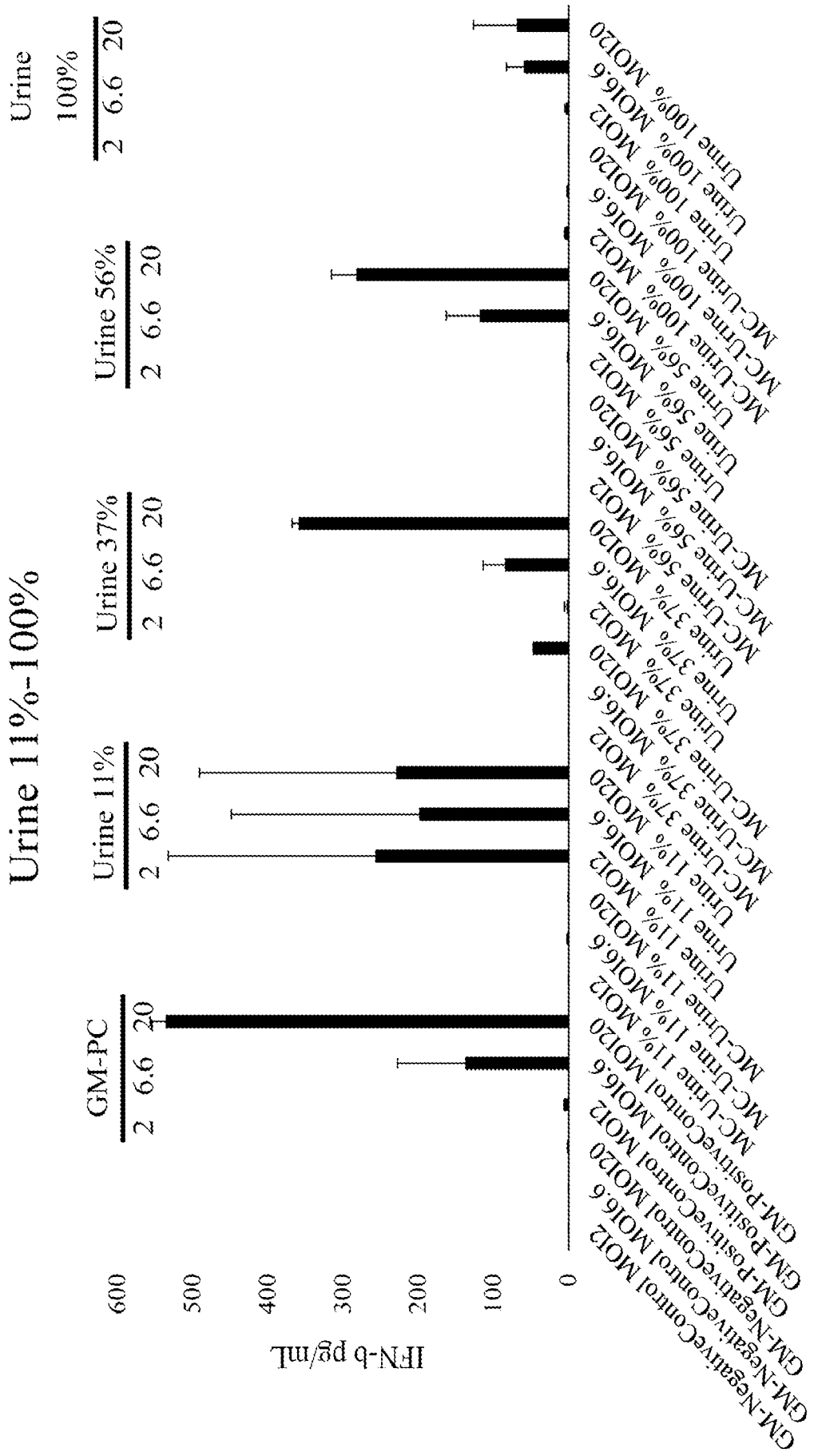
Urine 11%-100%
GM-PC
Urine 11%
Urine 37%
Urine 56%
Urine 100%
2 6.6 20
IFN-b pg/mL
600
500
400
300
200
100
0
GM-NegativeControl MOI2
GM-NegativeControl MOI6.6
GM-NegativeControl MOI20
GM-PositiveControl MOI2
GM-PositiveControl MOI6.6
GM-PositiveControl MOI20
MC-Urine 11% MOI2
MC-Urine 11% MOI6.6
MC-Urine 11% MOI20
MC-Urine 37% MOI2
MC-Urine 37% MOI6.6
MC-Urine 37% MOI20
MC-Urine 56% MOI2
MC-Urine 56% MOI6.6
MC-Urine 56% MOI20
MC-Urine 100% MOI2
MC-Urine 100% MOI6.6
MC-Urine 100% MOI20
Urine 11% MOI2
Urine 11% MOI6.6
Urine 11% MOI20
Urine 37% MOI2
Urine 37% MOI6.6
Urine 37% MOI20
Urine 56% MOI2
Urine 56% MOI6.6
Urine 56% MOI20
Urine 100% MOI2
Urine 100% MOI6.6
Urine 100% MOI20

FIG. 2D

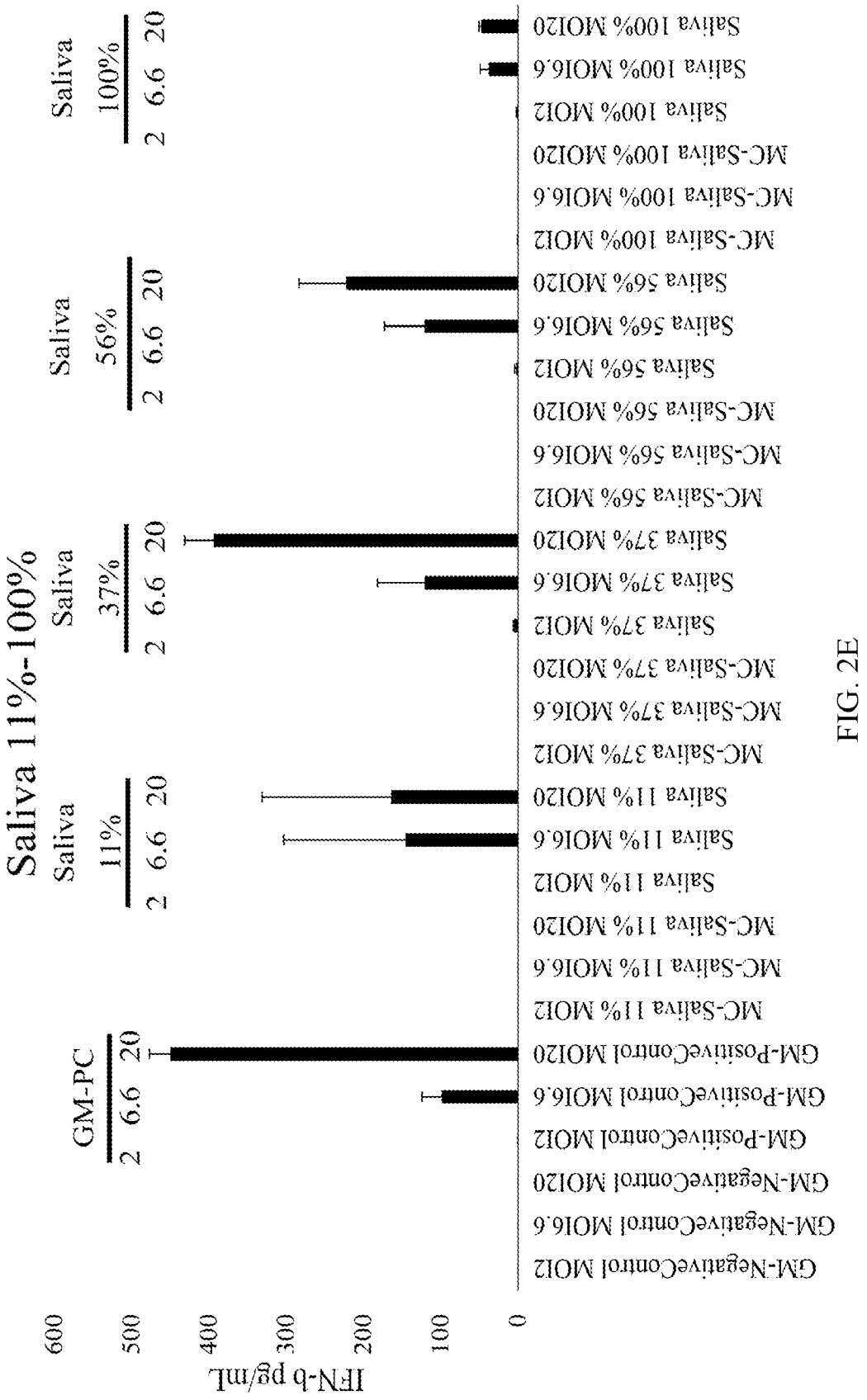
IFN-b pg/mL
600
500
400
300
200
100
0
GM-NegativeControl MOI2
GM-NegativeControl MOI6.6
GM-NegativeControl MOI20
GM-PositiveControl MOI2
GM-PositiveControl MOI6.6
GM-PositiveControl MOI20
MC-Saliva 11% MOI2
MC-Saliva 11% MOI6.6
MC-Saliva 11% MOI20
Saliva 11% MOI2
Saliva 11% MOI6.6
Saliva 11% MOI20
MC-Saliva 37% MOI2
MC-Saliva 37% MOI6.6
MC-Saliva 37% MOI20
Saliva 37% MOI2
Saliva 37% MOI6.6
Saliva 37% MOI20
MC-Saliva 56% MOI2
MC-Saliva 56% MOI6.6
MC-Saliva 56% MOI20
Saliva 56% MOI2
Saliva 56% MOI6.6
Saliva 56% MOI20
MC-Saliva 100% MOI2
MC-Saliva 100% MOI6.6
MC-Saliva 100% MOI20
Saliva 100% MOI2
Saliva 100% MOI6.6
Saliva 100% MOI20
GM-PC
2 6.6 20
Saliva 11%
2 6.6 20
Saliva 37%
2 6.6 20
Saliva 56%
2 6.6 20
Saliva 100%
2 6.6 20
Saliva 11%-100%

FIG. 2E

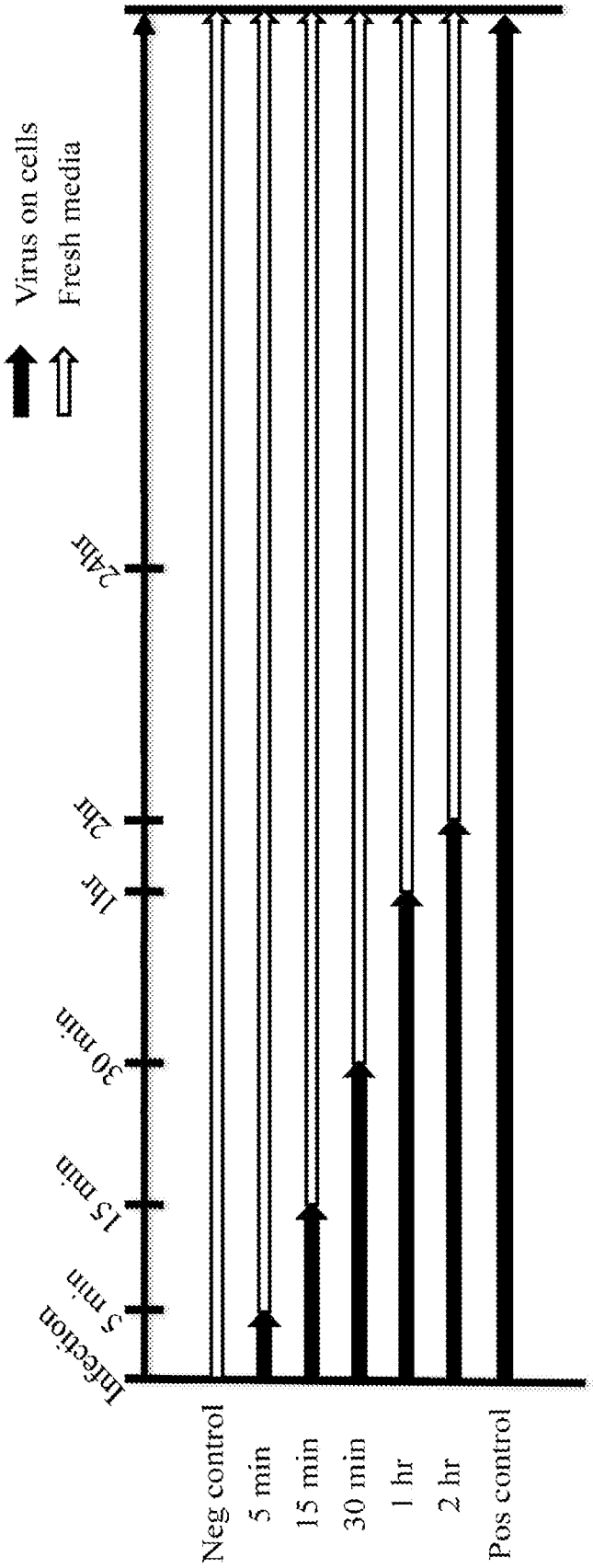
Virus on cells
Fresh media
Infection
5 min
15 min
30 min
1hr
2hr
24hr
Neg control
5 min
15 min
30 min
1 hr
2 hr
Pos control

FIG. 3A

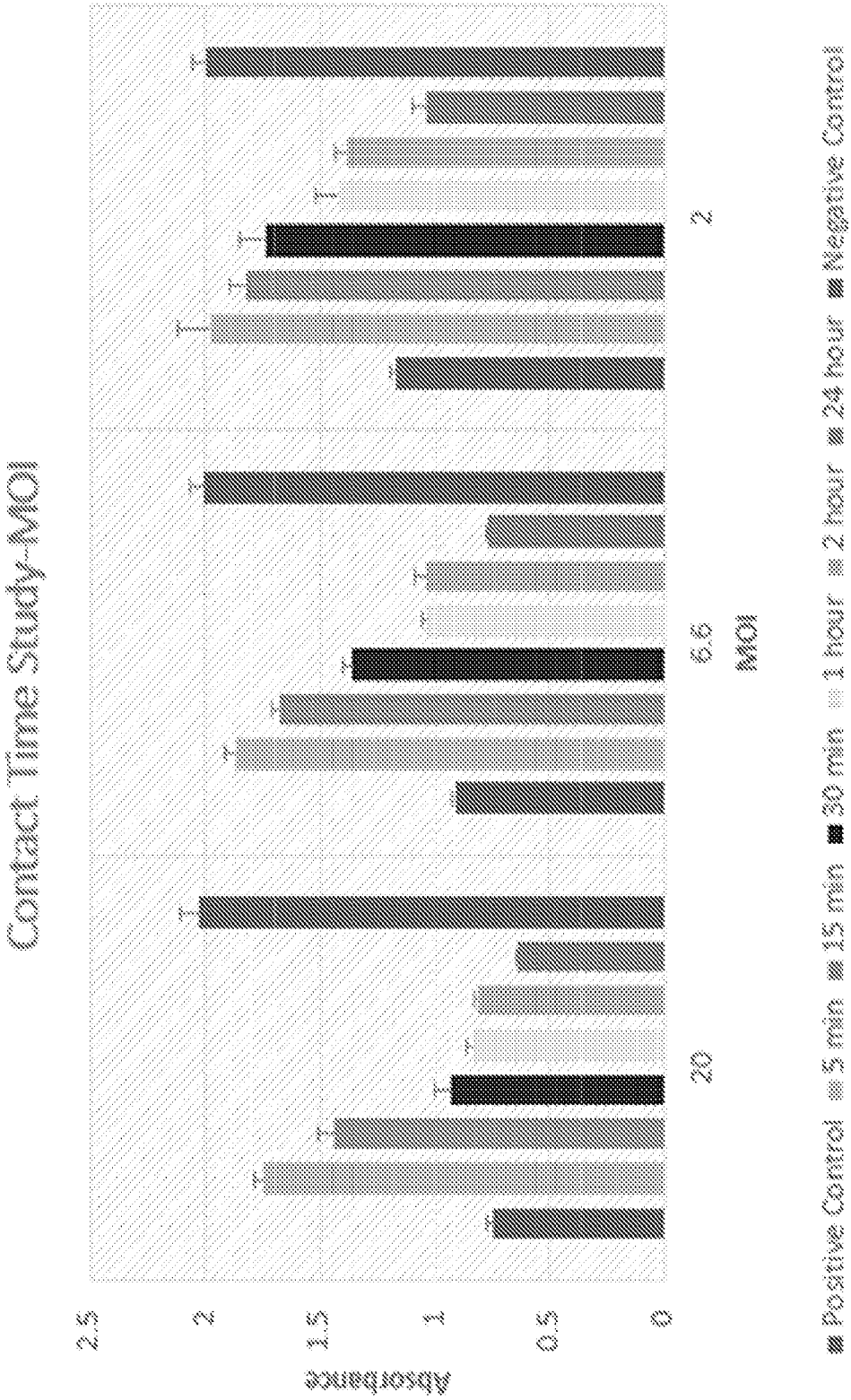
Contact Time Study-MOI
Absorbance
2.5
2
1.5
1
0.5
0
20
6.6
2
MOI
Positive Control
5 min
15 min
30 min
1 hour
2 hour
24 hour
Negative Control

FIG. 3B

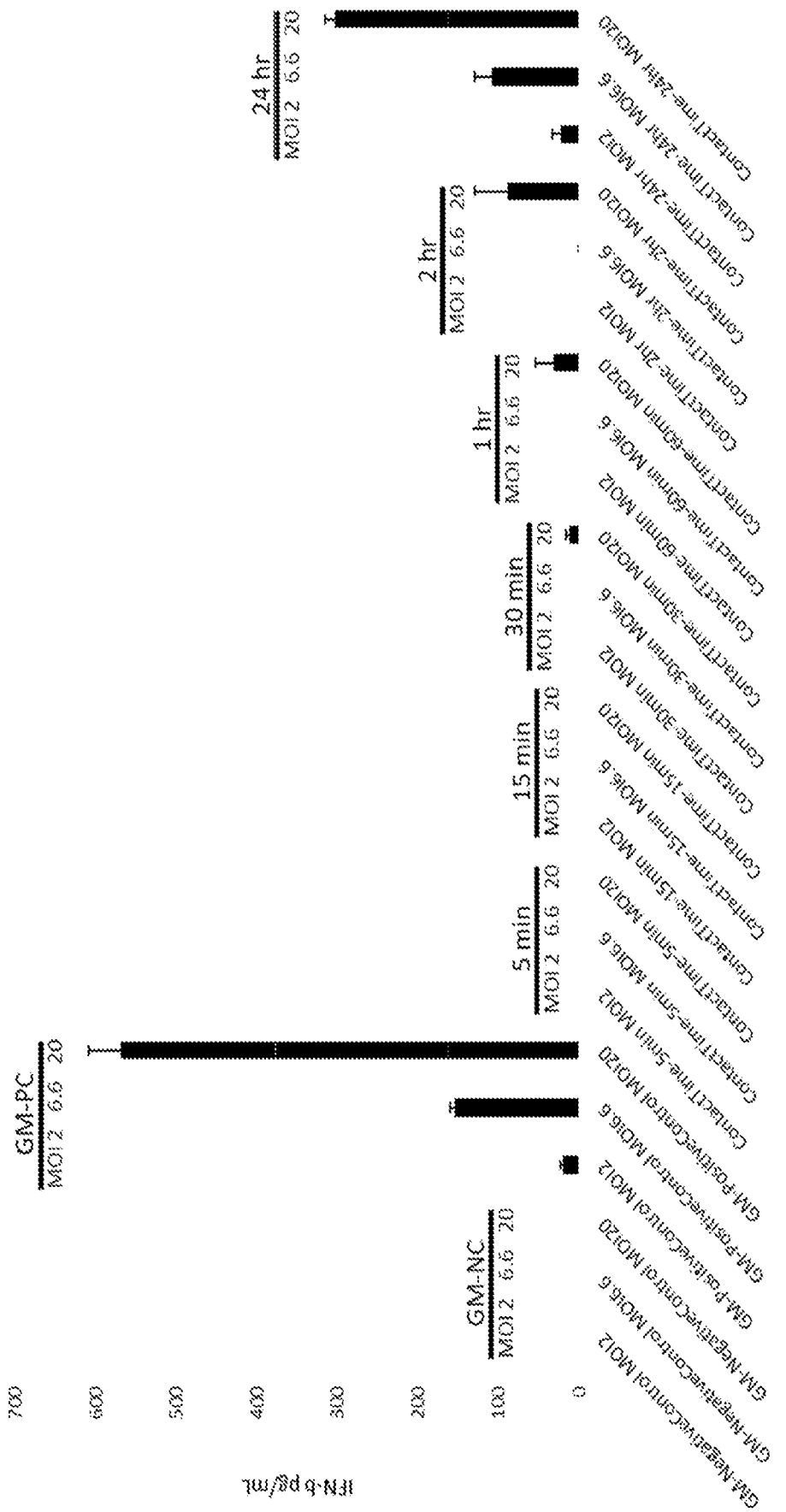
Contact Time IFN-β Release
IFN-b pg/mL
700
600
500
400
300
200
100
0
GM-PC
MOI 2 6.6 20
GM-NC
MOI 2 6.6 20
5 min
MOI 2 6.6 20
15 min
MOI 2 6.6 20
30 min
MOI 2 6.6 20
1 hr
MOI 2 6.6 20
2 hr
MOI 2 6.6 20
24 hr
MOI 2 6.6 20
GM-NegativeControl MOI2
GM-NegativeControl MOI6
GM-PositiveControl MOI20
GM-PositiveControl MOI2
GM-PositiveControl MOI6
Contact time-5min MOI20
Contact time-5min MOI2
Contact time-5min MOI6
Contact time-15min MOI20
Contact time-15min MOI2
Contact time-15min MOI6
Contact time-30min MOI20
Contact time-30min MOI2
Contact time-30min MOI6
Contact time-60min MOI20
Contact time-60min MOI2
Contact time-60min MOI6
Contact time-2hr MOI20
Contact time-2hr MOI2
Contact time-2hr MOI6
Contact time-24hr MOI20
Contact time-24hr MOI2
Contact time-24hr MOI6

FIG. 3C

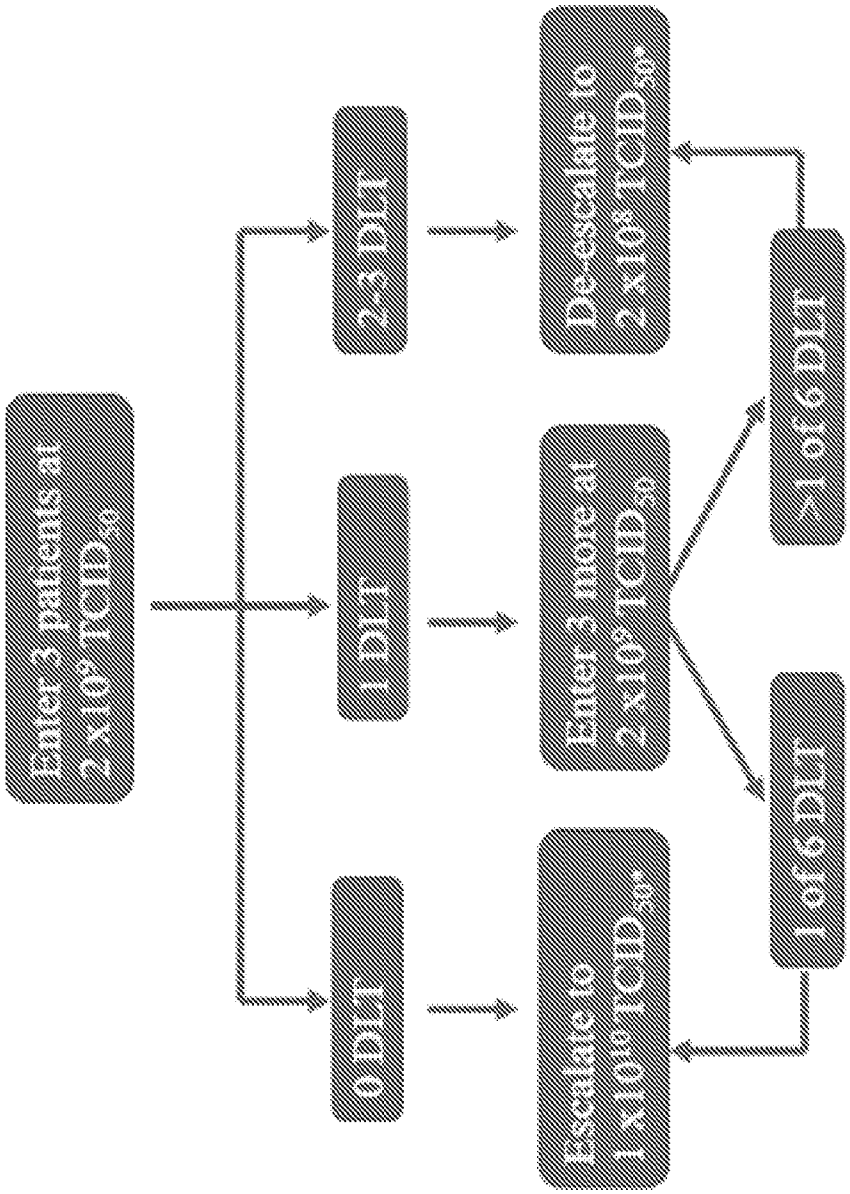
Enter 3 patients at 2 x10⁶ TCID₅₀
0 DLT
1 DLT
2-3 DLT
Escalate to 1 x10⁷ TCID₅₀*
Enter 3 more at 2 x10⁷ TCID₅₀
De-escalate to 1 x10⁷ TCID₅₀*
1 of 6 DLT
≥1 of 6 DLT
* To evaluate this dose, follow the same process

FIG. 4

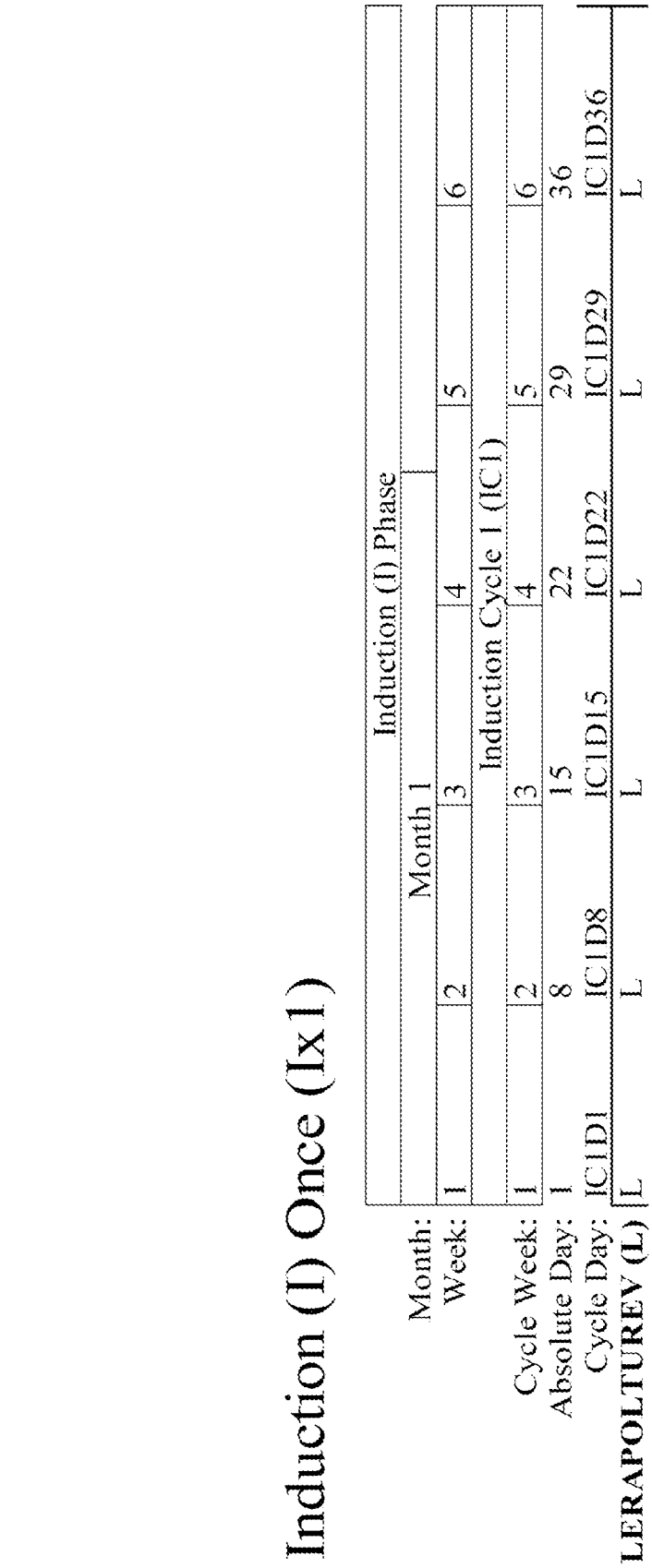
Induction (I) Once (Ix1)
Induction (I) Phase
Month 1
Induction Cycle 1 (IC1)
Month:
Week:
Cycle Week:
Absolute Day:
Cycle Day:
LERAPOLTUREV (L)
1
2
3
4
5
6
1
8
15
22
29
36
IC1D1
IC1D8
IC1D15
IC1D22
IC1D29
IC1D36
L
L
L
L
L
L

FIG. 5A

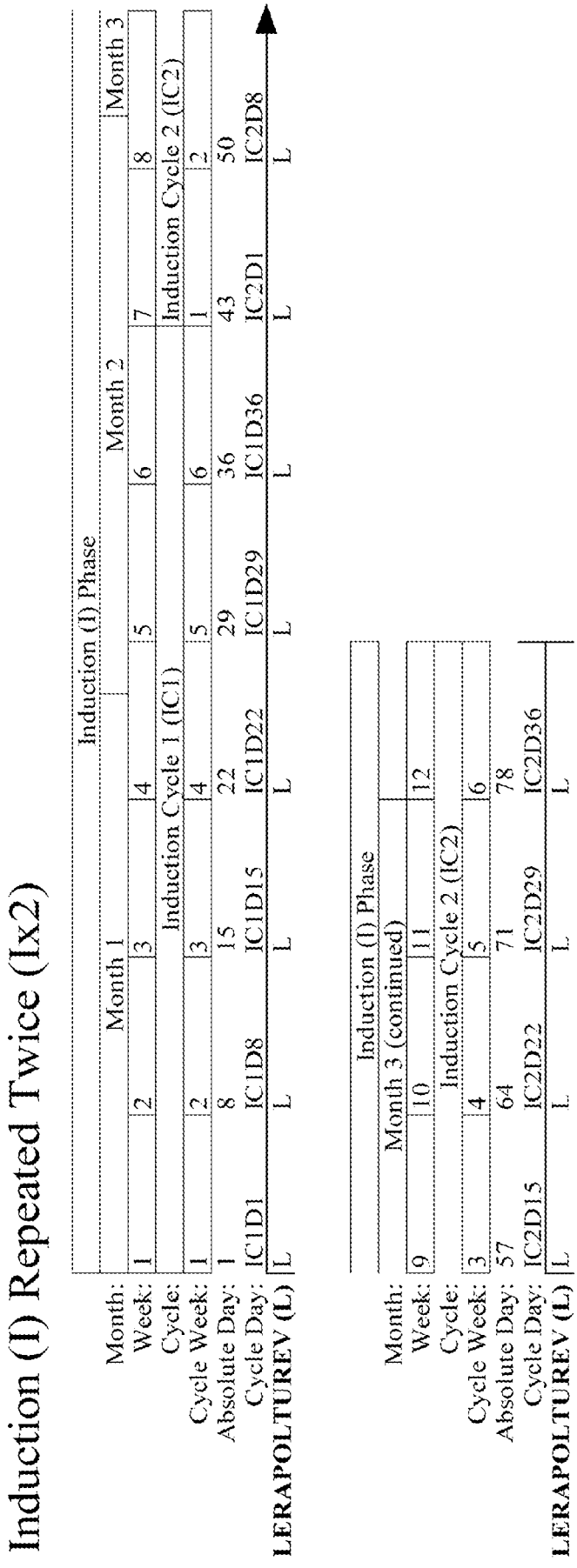
Induction (I) Repeated Twice (Ix2)
Induction (I) Phase
Month:
Month 1
Month 2
Month 3
Week:
1
2
3
4
5
6
7
8
Cycle:
Induction Cycle 1 (IC1)
Induction Cycle 2 (IC2)
Cycle Week:
1
2
3
4
5
6
1
2
Absolute Day:
1
8
15
22
29
36
43
50
Cycle Day:
IC1D1
IC1D8
IC1D15
IC1D22
IC1D29
IC1D36
IC2D1
IC2D8
LERAPOLTUREV (L)
L
L
L
L
L
L
L
L
Induction (I) Phase
Month:
Month 3 (continued)
Week:
9
10
11
12
Cycle:
Induction Cycle 2 (IC2)
Cycle Week:
3
4
5
6
Absolute Day:
57
64
71
78
Cycle Day:
IC2D15
IC2D22
IC2D29
IC2D36
LERAPOLTUREV (L)
L
L
L
L

FIG. 5B

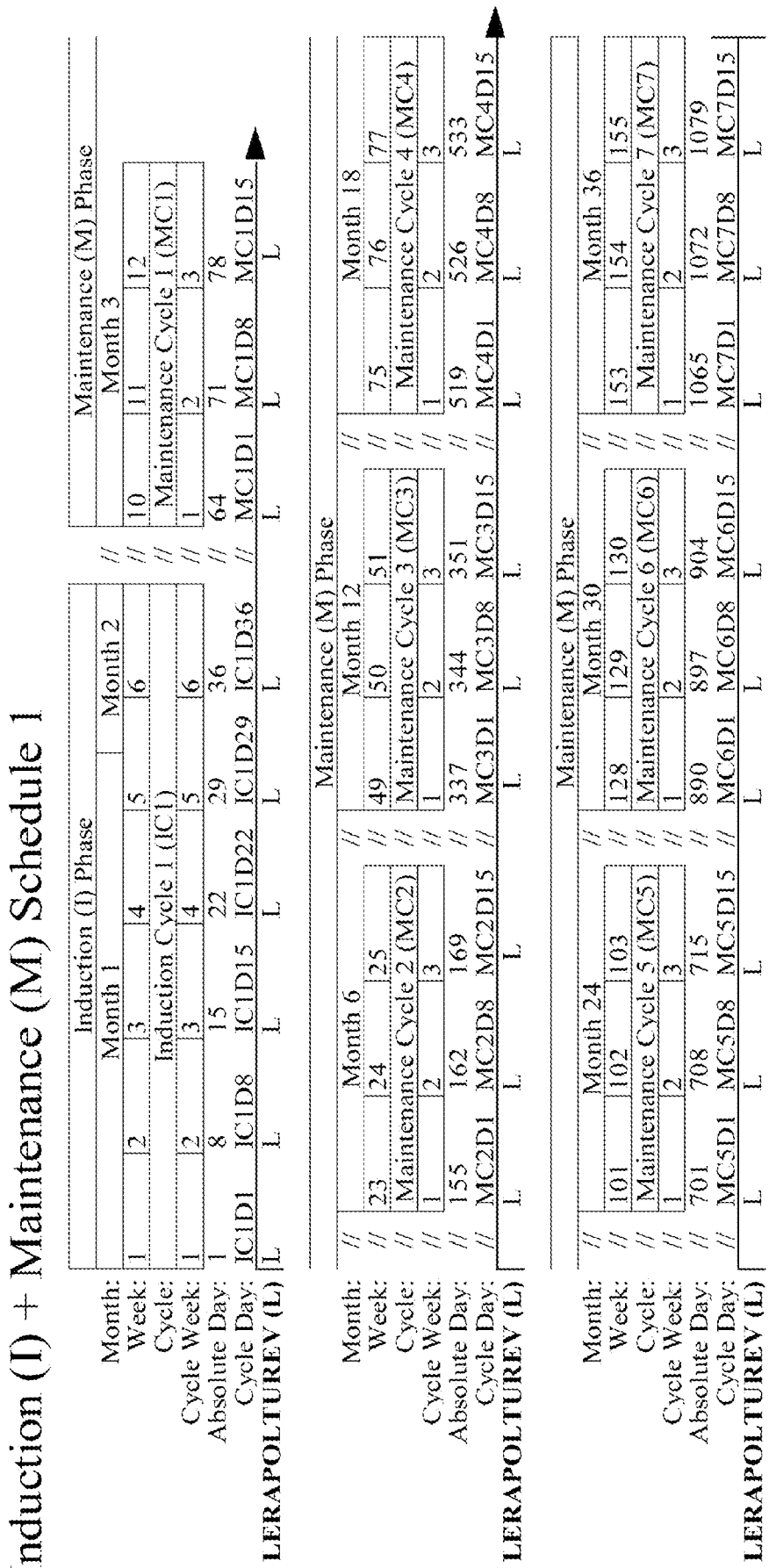
Induction (I) + Maintenance (M) Schedule 1
Induction (I) Phase
Maintenance (M) Phase
Month:
Week:
Cycle:
Cycle Week:
Absolute Day:
Cycle Day:
LERAPOLTUREV (L)
Month 1
Month 2
Month 3
Induction Cycle 1 (IC1)
Maintenance Cycle 1 (MC1)
IC1D1
IC1D8
IC1D15
IC1D22
IC1D29
IC1D36
MC1D1
MC1D8
MC1D15
Month 6
Month 12
Month 18
Maintenance Cycle 2 (MC2)
Maintenance Cycle 3 (MC3)
Maintenance Cycle 4 (MC4)
MC2D1
MC2D8
MC2D15
MC3D1
MC3D8
MC3D15
MC4D1
MC4D8
MC4D15
Month 24
Month 30
Month 36
Maintenance Cycle 5 (MC5)
Maintenance Cycle 6 (MC6)
Maintenance Cycle 7 (MC7)
MC5D1
MC5D8
MC5D15
MC6D1
MC6D8
MC6D15
MC7D1
MC7D8
MC7D15

FIG. 5C


Induction (I) + Maintenance (M) Schedule 2
Induction (I) Phase
Maintenance (M) Phase
Month:
Week:
Cycle:
Cycle Week:
Absolute Day:
Cycle Day:
LERAPOLTUREV (L)
Month 1
Month 2
Month 3
Month 6
Month 12
Month 18
Month 24
Month 30
Month 36
Induction Cycle 1 (IC1)
IC1D1
IC1D8
IC1D15
IC1D22
IC1D29
IC1D36
MC1D1
MC2D1
MC3D1
MC4D1
MC5D1
MC6D1
MC7D1
MC1
MC2
MC3
MC4
MC5
MC6
MC7

FIG. 5D