

SUMMARY

[0008] Local administration of an antimetabolite, such as gemcitabine, to the bladder leads to multiple biological effects, such as modulating immune responses in the tumor microenvironment, inducing systemic immunity and antigen presentation, and inducing cytotoxicity. Accordingly, the application discloses a method of treating urothelial carcinoma of the lower tract comprising administering to an individual an effective amount of an antimetabolite locally to the bladder.

[0008a] In one aspect, the present disclosure refers to use of an antimetabolite in the manufacture of a medicament for treating a urothelial carcinoma of the lower tract in an individual, wherein the antimetabolite is to be delivered locally to the bladder, wherein the urothelial carcinoma of the lower tract is muscle invasive bladder cancer.

[0008b] In one aspect, the present disclosure refers to use of an antimetabolite in the manufacture of a medicament for treating a urothelial carcinoma of the lower tract in an individual, wherein the antimetabolite is to be delivered locally to the bladder, wherein the urothelial carcinoma of the lower tract is non-muscle invasive bladder cancer.

[0008c] In one aspect, the present disclosure refers to use of a combination of an antimetabolite with a second agent in the manufacture of a medicament for treating a urothelial carcinoma of the lower tract in an individual, wherein the antimetabolite is to be delivered locally to the bladder.

[0009] In some examples, the method comprises a method of enhancing an immune response against a urothelial carcinoma of the lower tract of an individual comprising administering to the individual an effective amount of an antimetabolite, wherein the antimetabolite is delivered locally to the bladder.

[0010] In some examples, the method comprises a method of reducing recurrence or progression of a urothelial carcinoma of the lower tract in an individual comprising administering to the individual an effective amount of an antimetabolite, wherein the antimetabolite is delivered locally to the bladder.

[0011] In some examples, the method comprises a method of improving tumor microenvironment for cancer immunotherapy in an individual having a urothelial carcinoma of the lower tract, comprising administering to the individual an effective amount of an antimetabolite, wherein the antimetabolite is delivered locally to the bladder.

[0012] In some examples, the method comprises a method of sensitizing an individual having a urothelial carcinoma of the lower tract for radiation therapy, comprising administering to the individual an effective amount of an antimetabolite, wherein the antimetabolite is delivered locally to the bladder.

[0013] In some examples according to any of the methods described above, the method comprises a method of treating muscle invasive bladder cancer in an individual comprising

delivering an effective amount of gemcitabine locally to the bladder, wherein gemcitabine is delivered continuously for at least 24 hours.

[0014] In some examples according to any of the methods described above, the method comprises a method of bladder preservation in an individual comprising delivering an effective amount of gemcitabine locally to the bladder, wherein gemcitabine is delivered continuously for at least 24 hours.

[0014a] In one aspect, the present disclosure refers to use of an antimetabolite in the manufacture of a medicament for bladder preservation in an individual, wherein the antimetabolite is gemcitabine, wherein gemcitabine is to be delivered continuously to the bladder of the individual for 24 hours to 6 weeks, wherein the individual has a urothelial carcinoma of the lower tract.

[0015] In some examples according to any of the methods described above, the method comprises a method of treating non-muscle invasive bladder cancer in an individual comprising delivering gemcitabine locally to the bladder, wherein gemcitabine is delivered continuously for at least 24 hours.

[0016] In some examples according to any of the methods described above, the antimetabolite may be a nucleoside analog. In some of these examples, the antimetabolite is gemcitabine. In some examples according to any of the methods described above, the antimetabolite is delivered continuously into the bladder over a period of at least about 24 hours. In some examples according to any of the methods described above, the antimetabolite is delivered in a first phase of the delivery at a first dose followed by a second phase of delivery at a second dose. In some of examples, the first phase and the second phase are contiguous. Alternatively, in some examples, the first and second phase can be separated by a resting period.

[0017] In some examples according to any of the methods described above, the antimetabolite is delivered at a dose of from about 1 mg/day to about 300 mg/day. In some examples according to any of the methods described above, the concentration of antimetabolite in the urine is from about 0.1 µg/mL to about 200 µg/mL during the delivery period. In some of these examples according to any of the methods described above, the concentration of antimetabolite in the urine is about 10 µg/mL during the delivery period. In some examples according to any of the methods described above, the concentration of

antimetabolite in the plasma of the individual is less than about 1 $\mu\text{g/mL}$. In some examples according to any of the methods described above, the concentration of the antimetabolite is less than about 0.1 $\mu\text{g/mL}$. In some examples according to any of the methods described above, upon delivery of the antimetabolite, the ratio of antimetabolite in the urine to antimetabolite in the plasma of the individual is greater than about 500:1.

[0018] In some examples according to any of the methods described above, the antimetabolite is delivered over at least a month, wherein each antimetabolite delivery period is at least one day, and wherein the interval between each antimetabolite delivery period is no more than about a week. In some examples according to any of the methods described above, the interval between each antimetabolite period is 14 days. In some examples, there is no interval between each antimetabolite delivery period.

[0019] In some examples, the method comprises a) a first antimetabolite delivery period, wherein the concentration of antimetabolite in the urine of the individual is at least about 0.1 $\mu\text{g/mL}$; b) a rest period; and c) a second antimetabolite delivery period, wherein the concentration of antimetabolite in the urine of the individual is at least about 0.1 $\mu\text{g/mL}$ for at least a portion of the rest period. In some examples, the concentration of antimetabolite in the urine is greater than about 1.0 $\mu\text{g/mL}$ for at least about half of the rest period.

[0020] In some examples according to any of the methods described above, the method further comprises administering to the individual an effective amount of a second agent. In some of examples according to any of the methods described above, the second agent is delivered at the time the antimetabolite delivery is initiated. In some examples, the second agent is delivered before the antimetabolite is delivered. In some examples, the second agent is delivered after the antimetabolite delivery is initiated. In yet further examples, the second agent is delivered after the antimetabolite delivery is terminated.

[0021] In some examples according to any of the methods described above, the antimetabolite delivery period and the second agent delivery period may overlap with each

other. In some examples, the antimetabolite delivery period and the second agent delivery period may be non-overlapping.

[0022] In some examples according to any of the methods described above, the second agent is delivered systemically. In other examples, the second agent is delivered locally. In some examples, the second agent is delivered systemically in a first phase of the second agent delivery period, followed by a local delivery at a second phase of the second agent delivery period. In some examples, the second agent is delivered locally in a first phase of the second agent delivery period, followed by a systemic delivery at a second phase of the second agent delivery period. In some of examples, the first phase of the second agent delivery period and the second phase of the second agent delivery period are separated by at least about a month.

[0023] In some examples according to any of the methods described above, the antimetabolite and the second agent are delivered simultaneously. In some of these examples, the antimetabolite and the second agent are delivered via a single delivery device. In some examples according to any of the methods described above, the antimetabolite and the second agent are delivered at the same release rate. In other examples, the antimetabolite and the second agent are delivered at a different release rate.

[0024] In some examples, the second agent is delivered separately from the antimetabolite.

[0025] In some examples according to any of the methods described above, the second agent is delivered systemically. In some examples, the second agent is delivered locally.

[0026] In some examples according to any of the methods described above, the second agent is a chemotherapeutic agent. In some examples according to any of the methods described above, the chemotherapeutic agent is selected from the group consisting of paclitaxel, docetaxel, carboplatin, cisplatin, and oxaliplatin. In some examples, the second agent is an immunomodulating agent. In some examples, the immunomodulating agent is an immune

checkpoint inhibitor. In some examples, the immune checkpoint inhibitor is an inhibitor of an immune checkpoint protein selected from the group consisting of PD-L1, CTLA4, PD-L2, PD-1, B7-H3, B7-H4, HVEM, B- and T-lymphocyte attenuator (BTLA), Killer inhibitory receptor (KIR), GAL9, TIM3, A2AR, LAG-3, phosphatidylserine, CD27, TNF- α , CD33, Siglec-5, Siglec-7, Siglec-9, and Siglec-11. In some examples, the immunomodulating agent is an agonist of a costimulatory immune molecule. In some examples, the costimulatory immune molecule is selected from the group consisting of CD40, OX40, ICOS, CD28, CD137/4-1BB, CD27, IL-10, TGF-beta, TOR receptor, and glucocorticoid-induced TNFR-related protein GITR.

[0027] In some examples according to any of the methods above, the individual does not receive radiation therapy. In other examples, the method further comprises radiation therapy.

[0028] In some examples according to any of the methods above, the antimetabolite is delivered in a neoadjuvant setting. In some examples, the antimetabolite is delivered in an adjuvant setting. In some examples according to any of the methods described above, the antimetabolite is delivered in a pre-operative treatment. In some examples, the antimetabolite is delivered in a perioperative treatment.

[0029] In some examples according to any of the methods described above, the method further comprises a third therapy comprising surgery. In some of these examples, the delivery of the antimetabolite to the individual may be initiated at the time of surgery. In some examples according to any of the methods described above, delivery of the antimetabolite to the individual is initiated during a cystoscopy. In some examples according to any of the methods described above, delivery of the antimetabolite to the individual is initiated during a cystoscopy prior to surgery. In some examples, delivery of the antimetabolite to the individual is initiated during a cystoscopy following surgery.

[0030] In some examples according to any of the methods described above, the antimetabolite is delivered into the bladder by an intravesicular (intravesical) device. In some of these examples according to any of the methods described above, the intravesicular (intravesical) device comprises a housing configured for intravesicular (intravesical) insertion; and a dosage form comprising antimetabolite, wherein the housing holds the dosage form and is configured to release antimetabolite in an amount effective for the treatment of a urothelial carcinoma. In some examples according to any of the methods described above, the intravesicular (intravesical) drug delivery device comprises a housing which contains and controllably releases the antimetabolite and is elastically deformable between a retention shape configured to retain the device in the individual's bladder and a deployment shape for passage of the device through the individual's urethra. In some examples according to any of the devices described above, the device comprises a drug reservoir lumen bounded by a first wall and a second wall, wherein the first wall is impermeable to the drug and the second wall is permeable to the drug. In some examples according to any of the devices described above, the first wall and the second wall are adjacent one another and together form an annular tube defining the drug reservoir lumen. In some examples according to any of the devices described above, the second wall is in the form of a strip extending at least a portion of the length of the first wall structure. In some examples the first wall is cylindrical. In some examples, the second wall is disc-shaped. In some examples, the intravesicular (intravesical) drug delivery device comprises at least two drug reservoir lumens.

[0031] In some examples according to any of the methods described above, when a device is used to deliver the antimetabolite, the antimetabolite is released from the device by osmotic pressure. In some examples, the antimetabolite is released from the device by diffusion.

[0032] In some examples according to any of the devices described above, the antimetabolite contained in the housing is in a non-liquid form. In some of these examples, the non-liquid form is selected from the group consisting of tablets, granules, semisolids, powders, capsules, and combinations thereof.

[0033] In some examples according to any of the methods described above, the method provided herein comprises a method of treating a urothelial carcinoma of the lower tract, wherein the urothelial carcinoma is bladder cancer. In some examples, the bladder cancer is locally advanced bladder cancer or metastatic bladder cancer. In some examples, the bladder cancer is muscle invasive bladder cancer. In some example, the bladder cancer is non-muscle invasive bladder cancer. In some examples according to any of the methods described above, the bladder cancer is carcinoma in situ. In some examples, the bladder cancer is BCG (Bacillus Calmette-Guerin) refractory cancer or papillary bladder cancer. In some examples, the bladder cancer is BCG unresponsive cancer.

[0034] In some examples according to any of the methods described above, the method comprises administering to an individual an effective amount of an antimetabolite, wherein the individual is human. In some examples according to any of the methods described above, the individual is unfit for systemic therapy. In some examples according to any of the methods described above, the individual has a compromised immune system. In some examples according to any of the methods described above, the individual has a high level of an immune checkpoint protein. In some examples, the individual has a low level of an immune checkpoint protein. In some examples according to any of the methods described above, the individual has a high level of a nucleoside transporter. In some examples, the individual has a lower level of a nucleoside transporter.

[0035] In some examples according to any of the methods described above, the antimetabolite is gemcitabine and the method further comprises determining the gemcitabine/metabolite ratio in the urine, wherein a ratio below a threshold value is indicative of effective treatment.

[0036] In some examples, provided herein are kits for treating a urothelial carcinoma of the lower tract in an individual comprising: a) an antimetabolite and b) a second agent, wherein the antimetabolite is in a device for local delivery to the bladder. In some of these

examples, the antimetabolite is gemcitabine. In some examples, the second agent is an immunomodulatory agent.

[0037] Also provided herein is a device for local delivery of an antimetabolite and a second agent to the bladder of an individual comprising a) an antimetabolite and b) a second agent. In some examples, the antimetabolite is gemcitabine. In some examples, the second agent is an immunomodulatory agent.

[0038] In some examples according to any of the devices described above, the intravesicular (intravesical) drug delivery device comprises a housing defining a reservoir; a first unit contained within the reservoir, the first unit comprising an antimetabolite; and a second unit contained within the reservoir in a position distinct from the first unit, wherein the second unit comprises a functional agent that facilitates in vivo release of the drug from the housing. In some examples, the functional agent is an osmotic agent, a drug solubilizing agent, or a combination thereof, and the housing includes at least one drug release orifice which is in fluid communication with the reservoir.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] FIG. 1 shows the tumor size, as determined by net average intensity, in rats treated with 90 $\mu\text{g/ml}$, 180 $\mu\text{g/ml}$, or 350 $\mu\text{g/ml}$ gemcitabine compared to control.

[0040] FIG. 2 shows the level of gemcitabine in bladder tissue following continuous delivery of gemcitabine for one week.

[0041] FIG. 3 shows the level of gemcitabine in rat bladder tissue layers following continuous delivery of gemcitabine.

[0042] FIG. 4 shows the level of activated and regulatory T cells present in the tumor microenvironment after perfusion with 90 or 180 $\mu\text{g/mL}$ gemcitabine.