Abstract:

Title: METHODS AND COMPOSITIONS FOR ENHANCING AN IMMUNE RESPONSE, BLOCKING MONOCYTE MIGRATION, AMPLIFYING VACCINE IMMUNITY AND INHIBITING TUMOR GROWTH AND METASTASIS

Provided are methods for enhancing an immune response, methods for decreasing monocyte recruitment to lymph nodes, and methods for amplifying vaccine immunity by administering to an individual various monocyte migration inhibitors alone or in combination, and optionally in conjunction with an antigen, vaccine, or anti-tumor preparation. The invention also provides related methods of inhibiting tumor growth and metastasis. In addition, related compositions comprising one or more monocyte migration inhibitors and optionally comprising antigens, vaccines, or anti-tumor preparations are provided, as are kits containing such compositions.
METHODS AND COMPOSITIONS FOR ENHANCING AN IMMUNE RESPONSE,
BLOCKING MONOCYTE MIGRATION, AMPLIFYING VACCINE IMMUNITY AND
INHIBITING TUMOR GROWTH AND METASTASIS

CROSS REFERENCE TO RELATED APPLICATIONS
This application claims priority to U.S. Provisional Application No. 61/771,738, filed on March 1, 2013, U.S. Provisional Application No. 61/771,744, filed on March 1, 2013, U.S. Provisional Application No 61/807,189, filed on April 1, 2013, and U.S. Provisional Application No 61/818,176, filed on May 1, 2013, each of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION
The present invention provides compositions and methods for use in inhibiting monocyte migration, enhancing an immune response, decreasing monocyte recruitment to lymph nodes, amplifying vaccine immunity, and reducing tumor growth or metastasis, such methods including administering to an individual various monocyte migration inhibitors alone or in combination, and optionally in conjunction with an antigen, vaccine, anti-tumor preparation, and/or other agent. The present invention also includes kits comprising one or more monocyte migration inhibitors, alone or in combination with an antigen, vaccine, anti-tumor preparation, and/or other agent.

BACKGROUND OF THE INVENTION
Vaccine adjuvant-induced inflammation augments vaccine immunity in part by recruiting antigen presenting myeloid cells (monocytes and neutrophils) to vaccine draining lymph nodes (LNs) (Serafini et al. (2004) Cancer Res. 64:6337-6343; Martino et al. (2010) J. Immunol. 184: 2038-2047). However, recent evidence indicates that monocyte recruitment to LNs suppresses local B cell and T cell activation and proliferation (Mitchell et al. (2012) J. Immunology 189: 5612-5621; Mitchell et al. (2012) Int. Immunopharmacol. 15: 357-363). Lowered immune responses following vaccination can lead to decreased vaccine efficacy.
Moreover, the role of immature and immune suppressive myeloid cells, including neutrophils, monocytes, and tumor-associated macrophages, in promoting the growth of primary tumors is well established. Additionally, myeloid cells, and especially monocytes play an important role in creating favorable conditions for the seeding and growth of tumor metastases in the lungs, in part by establishing the so-called metastatic niche. Inflammatory monocytes recruited in response to tumor-derived signals have been shown to play a key role in promoting the growth of tumor metastases. The major chemokine regulating monocyte recruitment is MCP-1 (CCL2), which signals primarily via activation of the receptor CCR2 expressed principally on inflammatory monocytes.

There is clearly a need in the art for methods for administering vaccines and related vaccine compositions that augment vaccine efficacy by inhibiting the suppressive effects of monocytes at LNs and enhancing B cell and T cell responses. There is also a need in the art for methods for inhibiting the migration of inflammatory monocytes to the LNs, where they can suppress B and T cell responses. In addition, there is a need in the art for methods for inhibiting the migration of myeloid cells, and in particular inflammatory monocytes, to the site of tumors where they act to promote tumor growth and metastasis. The present invention is directed to overcoming these deficiencies in the art.

**BRIEF SUMMARY OF THE INVENTION**

In one embodiment, the present invention provides a method of inhibiting monocyte migration in an individual, the method comprising administering to the individual an antigen, vaccine, or an anti-tumor preparation in conjunction with an angiotensin II receptor blocker (ARB) or a compound of Formula (I):

![Chemical Structure](image)

(I)

wherein

R^1 is hydrogen or C_{1-6} alkyl;
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=== is a single bond or double bond;
Q\(^1\) is N or CH;
R\(^2\) is selected from hydrogen and \(\text{Ci}_{-6}\) alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO\(^2\)-, -NR\(^n\), or -CO-; wherein R\(^3\) is hydrogen or \(\text{Ci}_{-6}\) alkyl; and
R\(^3\) is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \(\text{Ci}_{-6}\) alkyl;
or pharmaceutically acceptable salts thereof. In certain embodiments, the monocytes are inflammatory monocytes or CD14\(^{\text{hi}}\)/CD16\(^-\) human monocytes.

In one related embodiment, the present invention provides a method of enhancing an immune response against an antigen in an individual, the method comprising administering to the individual an antigen in conjunction with an angiotensin II receptor blocker (ARB) or a compound of Formula (I).

In certain embodiments of any of the methods described herein, the enhanced immune response comprises an enhanced humoral immune response. In certain embodiments, the enhanced humoral immune response comprises an increased antibody titer against the antigen. In certain embodiments, the enhanced immune response comprises an enhanced cellular immune response. In certain embodiments, the enhanced cellular immune response comprises increased release of IFNy in response to the antigen. In certain embodiments, the enhanced immune response comprises an enhanced humoral immune response and an enhanced cellular immune response.

In another related embodiment, the present invention provides a method of decreasing recruitment of monocytes to a lymph node in an individual, the method comprising administering to the individual an antigen, vaccine, or an anti-tumor preparation in conjunction with an angiotensin II receptor blocker (ARB) or a compound of Formula (I). In certain embodiments, the monocytes are inflammatory monocytes or CD14\(^{\text{hi}}\)/CD16\(^-\) human monocytes. In certain embodiments, the lymph node is a draining lymph node. In certain embodiments, the draining lymph node is a vaccine draining lymph node.

In a further related embodiment, the present invention provides a method of amplifying vaccine immunity in an individual, the method comprising administering to the
individual an antigen or vaccine in conjunction with an angiotensin II receptor blocker (ARB) or a compound of Formula (I).

In yet another related embodiment, the present invention provides a method of inhibiting tumor growth or metastasis in an individual, the method comprising administering to the individual an anti-tumor preparation in conjunction with an angiotensin II receptor blocker (ARB) or a compound of Formula (I). In certain embodiments, the individual has a tumor or cancer.

In certain embodiments of any of the methods, compositions or kits described herein, the cancer is an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor. In some embodiments, the method further comprises administering a receptor tyrosine kinase inhibitor to the individual in combination with the ARB or the compound of Formula (I). In one embodiment, the TKI inhibitor is sunitinib or GW2580.

In particular embodiments of any of the methods, compositions and kits described herein, the compound of Formula (I) is a compound of Formula (II), Formula (III), Formula (IV) or Formula (V) as defined herein. In certain embodiments, the compound of Formula (I) is Ondansteron or Alosetron. In some embodiments, the compound of Formula (I) is Ondansetron. In some embodiments, the Ondansetron is administered at a dosage of 3 mg/kg. In some embodiments, the Ondansetron is administered at a dosage of less than 12 mg. In some embodiments, the compound of Formula (I) is Alosetron. In some embodiments, the Alosetron is administered at a dosage of less than 0.5 mg.

In certain embodiments of any of the methods, compositions and kits described herein, the ARB is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan. In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of 30 mg/kg. In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of less than 25 mg. In certain embodiments, the ARB is Candesartan, and the Candesartan is administered at a dosage of less than 4 mg. In certain embodiments, the ARB is Eprosartan, and the Eprosartan is administered at a dosage of less than 400 mg. In certain embodiments, the ARB is Irbesartan, and the Irbesartan is
administered at a dosage of less than 150 mg. In certain embodiments, the ARB is Olmesartan, and the Olmesartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Telmisartan, and the Telmisartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Valsartan, and the Valsartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Azilsartan, and the Azilsartan is administered at a dosage of less than 80 mg.

In certain embodiments, any of the methods described herein further comprise administering a receptor tyrosine kinase inhibitor to the individual in combination with the ARB or the compound of Formula (I). In one embodiment, the TKI inhibitor is suitinib or GW2580.

In certain embodiments comprising administering an ARB, the individual receiving an ARB does not have hypertension, congestive heart failure, a history of myocardial infarction, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB has not taken the ARB for the treatment of hypertension, congestive heart failure, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB does not have a detectable level of the ARB in their blood or urine prior to administration of the ARB in conjunction with the antigen.

In certain embodiments comprising administering Ondansetron or Alosetron, the individual has not taken the Ondansetron or the Alosetron for the treatment of irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONY), radiation-induced nausea and vomiting (RINV), or chemotherapy-induced nausea and vomiting (CINV). In some embodiments, the individual does not have a detectable level of the Ondansetron or the Alosetron in their blood or urine prior to administration of the Ondansetron or the Alosetron in conjunction with the antigen.

In certain embodiments of any of the methods, compositions or kits described herein, the antigen, vaccine or anti-tumor preparation and the ARB or the compound of Formula (I) are present in a single pharmaceutical composition. In certain embodiments, the single pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously,
intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

In certain embodiments of any of the methods, compositions and kits described herein, the ARB or the compound of Formula (I) is present in a first pharmaceutical composition and the antigen, vaccine or anti-tumor preparation is present in a second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the second pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the first pharmaceutical composition is administered before the second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered after the second pharmaceutical composition. In certain embodiments, the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another. In certain embodiments, the first pharmaceutical composition and the second pharmaceutical compositions are administered simultaneously.

In certain embodiments of any of the methods, compositions or kits described herein, the antigen comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

In certain embodiments of any of the methods, compositions or kits described herein, the vaccine comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.
In some embodiments, the present invention provides a method of inhibiting monocyte migration in an individual, the method comprising administering to the individual at least two monocyte migration inhibitors (MMIs). In some embodiments, the MMIs inhibit monocyte migration via different mechanisms of action. In some embodiments, the MMIs are selected from: Class I MMIs, which have angiotensin receptor blocking activity; Class II MMIs, which have serotonin receptor antagonist activity; and Class III MMIs, which have CCR2 receptor antagonist activity. In some embodiments, the Class I MMI is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan. In some embodiments, the Class II MMI is a compound of Formula (I). In some embodiments, the class II MMI compound of Formula (I) is a compound of Formula (II), Formula (III), Formula (IV), or Formula (V). In some embodiments, the Class III MMI is selected from the group consisting of competitive antagonists of the CCR2 receptor, RSI 02895, and RS504393.

In certain embodiments, the administration of the two or more MMI's having different mechanisms of action for inhibiting monocyte migration results in an additive inhibition of monocyte migration. In certain embodiments, the administration of the two or more MMI's having different mechanisms of action for inhibiting monocyte migration results in a synergistic inhibition of monocyte migration.

In some embodiments, two or more MMIs are administered to the individual simultaneously, and in other embodiments two or more MMIs are administered in conjunction with one another. As used herein, in reference to the administration of the two or more agents (e.g., two or more MMIs), the term "combined," or reference to the administration of the two or more agents (e.g., MMIs) "in combination," is meant to encompass both of these embodiments, i.e., the administration of the agents simultaneously or in conjunction with one another.

In some embodiments, a Class I MMI and a Class II MMI are administered in combination to an individual. In some embodiments, administration of a Class I MMI in combination with a Class II MMI results in synergistic inhibition of monocyte migration. In one certain embodiment, administration of ondansetron in combination with irbesartan results in a synergistic inhibition of monocyte migration inhibition. In one certain
embodiment, administration of ondansetron in combination with telmisartan results in a synergistic inhibition of monocyte migration inhibition. In one certain embodiment, administration of ondansetron in combination with Losartan results in a synergistic inhibition of monocyte migration inhibition.

In some embodiments, a Class I MMI and a Class III MMI are administered in combination to an individual. In some embodiments, administration of a Class I MMI in combination with a Class III MMI results in synergistic inhibition of monocyte migration. In one certain embodiment, administration of RS102895 in combination with irbesartan results in a synergistic inhibition of monocyte migration inhibition. In one certain embodiment, administration of RS102895 in combination with telmisartan results in a synergistic inhibition of monocyte migration inhibition. In one certain embodiment, administration of RS102895 in combination with Losartan results in a synergistic inhibition of monocyte migration inhibition.

In some embodiments, a Class II MMI and a Class III MMI are administered in combination to an individual. In one certain embodiment, RS102895 is administered in combination with ondansetron.

In some embodiments, the present invention provides a method of suppressing tumor growth or metastasis in an individual with cancer, the method comprising administering to the individual one or more MMI, e.g., one or more MMIs selected from Class I MMIs, Class II MMIs, and Class III MMIs. In some embodiments, the Class I MMIs are selected from the group consisting of losartan, irbesartan, and telmisartan; the Class II MMI is a compound of Formula (I), (e.g., Ondansetron, and Alosetron); and the Class III MMI is a competitive antagonists of the CCR2 receptor (e.g., RS102895, and RS504393). In some embodiments, the class II MMI compound of Formula (I) is a compound of Formula (II), Formula (III), Formula (IV), or Formula (V). In some embodiments, the method further comprises administering, in combination with the one or more MMI, a receptor tyrosine kinase inhibitor (TKI). In some embodiments, the TKI is sunitinib. In some embodiments, the TKI is GW2580. In some embodiments, administration of the TKI in combination with one or more MMIs results in an additive increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with a TKI or a MMI alone.
In some embodiments, administration of the TKI in combination with one or more MMIs results in a synergistic increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with a TKI or a MMI alone.

In some embodiments, the method of suppressing tumor growth or metastasis in an individual with cancer comprises administering to the individual two or more MMIs. In certain embodiments, at least two MMIs having different mechanisms of action for inhibiting monocyte migration are administered to the individual to suppress tumor growth or metastasis. In some preferred embodiments, two MMIs having different mechanisms of action for inhibiting monocyte migration are administered to the individual in conjunction with a TKI to suppress tumor growth or metastasis. In one embodiment, a Class I MMI, a Class II MMI, and a TKI, e.g., sunitinib, are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class I MMI, a Class III MMI, and a TKI, e.g., sunitinib, are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class II MMI, a Class III MMI, and a TKI, e.g., sunitinib, are administered in combination to suppress tumor growth or metastasis in an individual with cancer.

In one certain embodiment, ondansetron, irbesartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, ondansetron, telmisartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, irbesartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, telmisartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, ondansetron, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer.
In some embodiments, the present invention provides a composition comprising an MMI and an antigen or a vaccine. In some embodiments, the present invention provides a composition comprising two or more MMIs and an antigen or a vaccine.

In some embodiments, the present invention provides a composition comprising an MMI and an anti-tumor preparation. In some embodiments, the composition comprising an MMI and an anti-tumor preparation further comprises a TKI. In one certain embodiment, the TKI is sunitinib. In one certain embodiment, the TKI is GW2580. In some embodiments, the present invention provides a composition comprising two or more MMIs and an anti-tumor preparation. In some embodiments, the composition comprising two or more MMIs and an anti-tumor preparation further comprises a TKI. In one certain embodiment, the TKI is sunitinib.

In certain embodiments of any of the methods, compositions or kits described herein, the cancer is an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.

In some embodiments, compositions of the present invention further comprise a pharmaceutically acceptable carrier. In certain embodiments, the antigen or the vaccine in the composition comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide. In certain embodiments, the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan. In certain embodiments the compound of Formula (I) is Alosetron or Ondansetron.

In some embodiments, the invention provides a composition comprising (i) an antigen or a vaccine; and (2) an ARB or a compound of Formula (I). In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In certain embodiments, the antigen or the vaccine comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide. In certain embodiments, the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan. In certain embodiments the compound of Formula (I) is Alosetron or
Ondansetron.

In some embodiments, the invention provides a composition comprising an anti-tumor preparation and an ARB or a compound of Formula (I). In some embodiments, the composition further comprises a pharmaceutically acceptable carrier. In certain embodiments, the anti-tumor preparation comprises a therapeutic antibody, a topoisomerase inhibitor, an antimetabolite, a platinum-based agent, an alkylating agent, a tyrosine kinase inhibitor (TKI), an anthracycline antibiotic, an anti-angiogenic agent, or a vinca alkaloid. In certain embodiments, the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan. In certain embodiments the compound of Formula (I) is Alosetron or Ondansetron. In certain embodiments, the TKI is sunitinib.

The invention also provides kits comprising any of the compounds or compositions described herein and, optionally, instructions for use. In certain embodiments, kits of the invention comprise a vaccine, an antigen, or an anti-tumor preparation and an ARB or a compound of Formula (I) and, optionally, instructions for use. In certain embodiments, the ARB or the compound of Formula (I) is provided in a first container, and wherein the vaccine, antigen, or anti-tumor preparation is provided in a second container. In some embodiments of the kits, the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan. In some embodiments of the kits, the compound of Formula (I) is Alosetron or Ondansetron.

In certain embodiments, the invention provides a kit comprising two or more monocyte migration inhibitors (MMIs). In some embodiments, at least one of said two MMIs is described herein. In some embodiments, the invention provides a kit comprising at least two MMIs, wherein at least two of the MMIs are different classes of MMIs. In some embodiments, the invention provides a kit comprising at least two MMIs, wherein at least two of the MMIs have a different mechanism for action for inhibiting monocyte migration. In some embodiments, the invention provides a kit comprising at combination of at least two MMIs, wherein the combination comprises a first MMI and a second MMI, wherein the first MMI is a Class I MMI and the second MMI is a Class II MMI or a Class III MMI. In some embodiments, the Class II MMI is Ondansetron. In some embodiments, the Class III MMI is RSI 02895 or RS504393. In some embodiments, the Class I MMI is selected from the group
consisting of irbesartan, telmisartan, and losartan. In some embodiments, the invention provides a kit comprising at least two MMIs from different classes of MMIs and the kit further comprises an antigen, a vaccine, or an anti-tumor preparation. In certain embodiments, the at least two MMIs are present in the same composition, whereas in other embodiments, at least two of the MMIs are present in separate compositions. In particular embodiments, each composition is present in a separate container.

In some embodiments, the invention provides a kit comprising at least two MMIs, wherein at least two of the MMIs are a different classes of MMI, and the kit further comprises a receptor tyrosine kinase inhibitor (TKI). In one embodiment, the TKI is a broad spectrum tyrosine kinase inhibitor. In some embodiments, the tyrosine kinase inhibitor is sunitinib. In some embodiments, the kit comprising at least two MMIs, and optionally comprising a TKI, also optionally comprises an antigen, a vaccine, or an anti-tumor preparation. In particular embodiments, the kits further comprise a pharmaceutically acceptable carrier. In some embodiments, the kit of the present invention which comprises two or more MMIs and optionally a TKI and also optionally an antigen, a vaccine, or an anti-tumor preparation and also optionally a pharmaceutically acceptable carrier comprises one or more container or compartment, wherein the MMIs and the optional TKI, antigen, vaccine, anti-tumor preparation, and/or carrier are comprised either in separate containers or compartments, or they comprised a single compartment. In some embodiments, the compositions comprised in kits such as these further comprising instructions for use.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the results of experiments conducted to assess the ability of Losartan to inhibit human monocyte migration in vitro.

FIGURE 2 shows the results of experiments conducted to assess the ability of Losartan to inhibit canine monocyte migration in vitro.

FIGURE 3 shows the results of experiments conducted to assess the ability of Ondansetron to inhibit human monocyte migration in vitro.

FIGURE 4 shows the results of experiments conducted to assess the ability of RSI 02895 to inhibit murine monocyte migration in vitro.
FIGURE 5 shows the results of experiments conducted to determine the effect of Ondansetron treatment on the migration of immune suppressive inflammatory monocytes to lymph nodes following vaccination in mice.

FIGURE 6 provides a quantitative analysis of the results shown in FIGURE 5.

FIGURE 7 shows the results of experiments conducted to determine the effect of Losartan treatment on the migration of immune suppressive inflammatory monocytes to lymph nodes following vaccination in mice.

FIGURE 8 shows the results of experiments conducted to determine the effect of Losartan treatment on the migration of circulating immune suppressive inflammatory monocytes in healthy dogs. 8(a) and 8(b) show the results from two different dogs.

FIGURE 9 shows the results of experiments conducted to determine the effect of Losartan treatment on the migration of circulating immune suppressive inflammatory monocytes in dogs with cancer.

FIGURE 10 shows the results of experiments conducted to determine the effect of Losartan treatment on a humoral immune response.

FIGURE 11 shows the results of experiments conducted to determine the effect of Ondansetron treatment on a humoral immune response.

FIGURE 12 shows the results of experiments conducted to determine the effect of Losartan treatment on a cellular immune response.

FIGURE 13 shows the results of experiments conducted to determine the effect of Ondansetron on tumor vaccine responses.

FIGURE 14 shows the results of experiments conducted to determine the effect of Ondansetron treatment on myeloid cell accumulation in tumor tissues following vaccination.

FIGURE 15 shows a schematic demonstrating the establishment of CT26 colon cancer, and 4T1 breast cancer metastatic tumor cell models, as well as dosing routes and schedules utilized for the corresponding experiments. Briefly, on day one of the experiments CT26 or 4T1 tumor cells, expressing luciferase, were introduced into BALB/c mice via intravenous injection. Twenty-four hours later, experimental drug dosing was initiated via intraperitoneal (IP) injection, subcutaneous injection (SC), or oral administration in water.
FIGURE 16 shows the results of an experiment conducted to determine the effect of twice daily i.p. administration of the CCR2 antagonist RSI 02895 on tumor growth in mice using a metastatic colon carcinoma cell line CT26-induced tumors cell model. RSI 02895 treated mice showed significantly reduced tumor growth as compared to control mice (p<0.01).

FIGURE 17 shows the results of experiments conducted to determine the effect of the CCR2 antagonist RS504393 on tumor growth. CT26 lung metastases were established as described in Figure 15, and beginning twenty-four hours later, once daily RS504393 (5 mg/kg) dosing was initiated via IP injection. On day 14, in vivo tumor imaging was performed using the IVIS imaging system (Perkin Elmer, Akron Ohio, USA), as per manufacturers recommendations.

FIGURE 18 shows the results of experiments conducted to determine the effect of the CCR2 antagonist RS504393 on the survival of mice with CT26 lung metastases (n=5 mice / group). RS504393 administration and dosing was the same as is described in Figure 17. Treatment with RS504393 significantly prolonged survival (p=.01), with one mouse having an apparent cure.

FIGURE 19 shows the results of experiments conducted to determine the effect of the CCR2 antagonist RSI 02895 on monocyte infiltration into lungs with CT26 metastases (n=5 mice / group). RSI 02895 administration and dosing was the same as is described in Figure 17 with regard to RS504393. Treatment with RSI 02895 significantly suppressed inflammatory monocyte infiltration into lung tissues containing CT26 metastases (p=.05).

FIGURE 20 shows the results of experiments conducted to determine the effect of the CCR2 antagonist RSI 02895 on the growth of metastatic tumors. 4T1 breast cancer metastases were established as described in Figure 15, and beginning twenty-four hours later, once daily RSI 02895 (5 mg/kg) dosing was initiated via IP injection. On day 9, tumor imaging was performed using the IVIS imaging system (as described herein). RSI 02895-treated animals had significantly smaller tumors (as shown in Figure 21).

FIGURE 21 shows quantification of the images shown in Figure 20, demonstrating that RSI 02895 induced a significant suppression of tumor growth (p<0.05).
FIGURE 22 shows the results of experiments conducted to determine the effect of the CCR2 antagonist RSI 02895 on metastatic tumor growth. 4T1 breast cancer metastases were established as described in Figure 15, and beginning twenty-four hours later, RSI 02895 dosing was initiated by providing the mice *ad libitum* access to water dosed with the drug (5mg/kg/mouse/day based on an estimated consumption of 3 ml/day/mouse). Tumor metastasis was monitored by IVIS (as described herein). Mice receiving the RS drug had a significantly decreased growth of cancer cell metastases (p<0.05).

FIGURE 23 shows the results of experiments conducted to determine the synergistic interactions for the inhibition of monocyte migration resulting from the combination of Class I (irbesartan, telmisartan, losartan) and Class II (ondansetron) monocyte migration inhibitors. (A) shows a strong synergistic interaction with ondansetron and irbesartan co-treatment. (B) shows a strong synergistic interaction with ondansetron and telmisartan co-treatment. (C) shows a weak synergistic interaction with ondansetron and losartan co-treatment. Three asterisks indicates a p value greater than or equal to 0.001; two asterisks indicates a p value greater than or equal to p<0.01; one asterisk indicates a p value greater than or equal to <0.05.

FIGURE 24 shows the results of experiments conducted to determine the synergistic interactions for the inhibition of monocyte migration resulting from the combination of Class I (irbesartan, telmisartan, losartan) and Class III (RSI 02895) monocyte migration inhibitors. (A) shows a strong synergistic interaction with RSI 02895 and losartan co-treatment. (B) shows a weak or no synergistic interaction with RSI 02895 and telmisartan co-treatment. (C) shows a strong synergistic interaction with RSI 02895 and irbesartan co-treatment. Three asterisks indicates a p value greater than or equal to 0.001; two asterisks indicates a p value greater than or equal to p<0.01; one asterisk indicates a p value greater than or equal to <0.05.

FIGURE 25 shows the results of experiments conducted to determine if synergistic interactions for the inhibition of monocyte migration result from the combination of Class II (ondansetron) and Class III (RSI 02895) monocyte migration inhibitors. No interactions were observed when ondansetron and RSI 02895 were co-administered.
FIGURE 26 shows the results of an experiment conducted to determine the synergistic interactions between losartan and sunitinib for inhibiting tumor growth.

FIGURE 27 shows the results of preliminary experiments conducted to determine the synergistic interactions between losartan and sunitinib for inhibiting monocyte migration. 27(a) shows the percentage of all monocytes present in the lungs of the mice having lung metastases. 27(b) shows the percentage of PD-L1+ monocytes present in the lungs of the mice having lung metastases.

FIGURE 28 shows the results of preliminary experiments conducted to determine the effectiveness of combining RSI 02895 and CLDC (a nonspecific immunostimulant) for inhibiting primary tumor growth.

FIGURE 29 shows the results of preliminary experiments conducted to determine the effectiveness of combining RSI 02895 and CLDC (a nonspecific immunostimulant) for inhibiting the growth of metastatic tumors. Figure 29(a) shows tumor growth throughout the experiment, as measured by IVIS. Figure 29(b) is the same data as reported in Figure 29(a) zoomed in on the lower datapoints.

FIGURE 30 shows survival data from the experiment discussed in Figure 29, and demonstrates an increase in the survival of mice receiving RSI 02895 as compared to controls.

FIGURE 31 shows the results of experiments conducted to determine the effect of GW2580 treatment on a humoral immune response.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides, inter alia, compositions, methods, and kits related to monocyte migration inhibitors used for enhancing an immune response in an individual, decreasing the recruitment of monocytes to a lymph node in an individual, increasing or amplifying vaccine immunity in an individual, and inhibiting or reducing tumor growth or metastasis in an individual with cancer. Certain embodiments of the invention are based in part on the observation that compounds of Formula (I):
wherein

R
$^1$
 is hydrogen or C$i$–$^6$ alkyl;

Q
$^1$
 is N or CH;

R
$^2$
 is selected from hydrogen and C$i$–$^6$ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO$_2$-, -NR$_a$-, or -CO-; wherein R$^3$ is hydrogen or C$i$–$^6$ alkyl; and

R
$^3$
 is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C$i$–$^6$ alkyl;

or pharmaceutically acceptable salts thereof; which are typically indicated for treating nausea and vomiting, certain forms of psychosis, symptoms of severe irritable bowel syndrome, tardive dyskinesia, and forms of gastroenteritis, inhibit the migration of monocytes (e.g., migration to vaccine draining lymph nodes), inhibit the migration of monocytes, inhibit the suppressive effects of monocytes on vaccine immunity, and inhibit the pro-metastatic actions of inflammatory monocytes, and inhibit tumor growth and metastasis.

In certain embodiments, the monocyte migration inhibitors are administered in combination with a receptor tyrosine kinase inhibitor (TKI). In some embodiments, the TKI is a broad spectrum TKI. In one embodiment, the TKI is sunitinib or GW2580.

Certain embodiments of the invention are based in part on the observation that angiotensin II receptor blockers (ARBs), which are typically indicated for the treatment of hypertension, inhibit the migration of monocytes (e.g., migration to vaccine draining lymph nodes), inhibit the suppressive effects of monocytes on vaccine immunity, and inhibit tumor growth and metastasis.

The invention also provides, inter alia, compositions, kits, and methods related to the use of combinations of two or more monocyte migration inhibitors, such as CCR2 antagonists, ARBs, and compounds of Formula (I) as defined herein, to increase or enhance
an immune response, to inhibit migration of monocytes (e.g., to inhibit or reduce recruitment of monocyte to a draining lymph node e.g., a vaccine draining lymph node), to amplify or increase vaccine immunity, and to inhibit or reduce tumor growth or metastasis. In some embodiments, such combinations show improved inhibition of monocyte migration, and in some instances result in synergistic inhibition of monocyte migration, thus providing potentially superior methods of enhancing an immune response in an individual, decreasing the recruitment of monocytes to a lymph node in an individual, amplifying vaccine immunity in an individual, and reducing tumor growth or metastasis in an individual with cancer.

The present invention also provides, inter alia, compositions, kits, and methods related to the use of monocyte migration inhibitors as a anti-metastatic agents. These embodiments are based in part on the discovery that the monocyte migration inhibitors as disclosed herein can inhibit tumor growth and metastasis. Moreover, use of such compositions in combination with other anti-tumor / anti-metastatic agents (e.g., receptor tyrosine kinase inhibitors such as sunitinib) results in the synergistic inhibition of monocyte infiltration into tumor bearing tissue, and a resultant synergistic decrease in tumor growth.

GENERAL METHODS

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, certain illustrative methods and materials are described. For the purposes of the present invention, the following terms are defined below.

As used herein, the singular form "a", "an", and "the" includes plural references unless indicated otherwise.

As used herein, the term "about," when used in reference to a value or parameter refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "about" a value or parameter herein includes (and describes) aspects that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X." In some embodiments, about refers to a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight, length, or other parameter that varies by as much as 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight, length, or other parameter.

As used herein, the term "effective amount" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result, e.g., an enhanced immune response to an antigen, a decrease in monocyte recruitment to a lymph node following vaccination, an amplification of vaccine immunity, an inhibition of tumor growth and metastasis, etc. An effective amount can be provided in one or more administrations.

As used herein, the term "in conjunction with" refers to the temporal property of two or more events occurring at approximately the same time, e.g., between 0-12 hours of one
another. For example, when a compound of Formula (I) is administered in conjunction with an antigen or a vaccine, the compound of Formula (I) and the antigen or vaccine are administered within less than a 12 hour period. The term "in conjunction with" is not limited by the order in which the two or more events occur. Furthermore, the term in conjunction with does not exclude the two events from occurring simultaneously. For example, when an ARB is administered in conjunction with an antigen or a vaccine, the ARB and the antigen or the vaccine may optionally be administered simultaneously, e.g., as a mixture; or they may be administered separately in conjunction with one another.

As used herein, the term "individual" refers to a mammal, e.g., a human, a companion animal (e.g., dog, cat, rodent, rabbit, etc.), a sport animal (e.g., horse, dog, bull, etc.), a farm or food animal (e.g., pig, cow, sheep, goat, etc.), livestock (e.g., donkeys, goats, guinea pigs, sheep, cattle, llamas, etc.), or any other mammalian veterinary animal.

As used herein, the term "inhibiting" means negatively modulating, i.e., decreasing or reducing that which is being inhibited. For example, 'inhibiting monocyte migration" means decreasing or reducing monocyte migration. Typically, inhibiting refers to a statistically significant negative modulation.

As used herein, the term "mixed function" means an inhibitor that shows broad specificity. This term is used interchangeably with the term "broad spectrum". In certain embodiments, a TKI inhibitor is a mixed function or broad spectrum TKI inhibitor such as, e.g., sunitinib or GW2580.

As used herein, the term "modulating" means changing, and includes positive modulating, such as "increasing," "enhancing" or "stimulating," as well as negative modulating such as "decreasing" or "reducing," typically in a statistically significant or a physiologically significant amount as compared to a control. An "increased," "stimulated" or "enhanced" amount is typically a "statistically significant" amount, and may include an increase that is 1.1, 1.2, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (e.g., 500, 1000 times) (including all integers and decimal points in between and above 1, e.g., 1.5, 1.6, 1.7, 1.8, etc.) the amount produced by no composition (e.g., in the absence of any of the monocyte migration inhibitors disclosed herein) or a control composition, sample or test subject. A "decreased" or "reduced" amount is typically a "statistically significant" amount,
and may include a 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%,
15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,
75%, 80%, 85%, 90%, 95%, or 100% decrease in the amount produced by no composition
(the absence of an agent or compound) or a control composition, including all integers in
between.

By "statistically significant," it is meant that the result was unlikely to have occurred
by chance. Statistical significance can be determined by any method known in the art.
Commonly used measures of significance include the p-value, which is the frequency or
probability with which the observed event would occur, if the null hypothesis were true. If
the obtained p-value is smaller than the significance level, then the null hypothesis is
rejected. In simple cases, the significance level is defined at a p-value of 0.05 or less.

It is understood that aspects and embodiments of the invention described herein
include "comprising," "consisting," and "consisting essentially of" aspects and
embodiments.

COMPOSITIONS OF THE INVENTION

During vaccination, an antigen is administered to an individual to stimulate antigen-
specific immunity, thus increasing the individual's resistance (or decreasing his or her
susceptibility) to a particular disease or infection. In order for vaccination to be effective, the
antigen must be capable of provoking robust humoral (i.e., B cell) and/or cell-mediated (i.e.,
T cell) immune responses. However, CCR2+ inflammatory monocytes, which are recruited
to the site of vaccination and to the vaccine draining lymph nodes, have been shown to have
a suppressive effect on B cell and T cell responses (Mitchell et al. (2012) J. Immunology
189: 5612-5621).

In certain embodiments, the present invention relates to monocyte migration
inhibitors (MMI), and various uses thereof. Several monocyte migration inhibitors (MMIs)
are known or disclosed in the present invention. These include, but are not limited to Class I
inhibitors, as disclosed herein, Class II inhibitors, as disclosed herein, Class III inhibitors, as
disclosed herein.
Class I MMIs include, e.g., but are not limited to, various angiotensin II receptor blocking drugs (ARBs) such as losartan, irbesartan, telmisartan, and others as disclosed herein, that each exert MMI activity.

Class II MMIs include, e.g., but are not limited to, the anti-nausea drug ondansetron (a serotonin receptor antagonist), and other related compounds of Formula (I), as disclosed herein, that also exert MMI activity.

Class III MMIs include CCR2 receptor antagonists, e.g., including the specific CCR2 antagonist compounds disclosed herein which were specifically designed as MMIs, and function as pure antagonists of the CCR2 receptor on monocytes. Two examples (RS102895, and RS504393) of class III drugs are listed here, but there are many others in this same family that share a mechanism of activity (competitive antagonists for CCR2) all of which are suitable for use in the present invention.

It is to be understood that in certain embodiments, all MMIs are acceptable for use in the present invention. Accordingly, the examples provided herein are for illustrative purposes, and are not to be considered limiting with respect to the MMI that is used.

In various embodiments, the MMIs are used in combination with other agents, such as, e.g., Liposomal Bisphosphonates (e.g., Clodronate) and various TKI inhibitors that are able to inhibit monocyte migration or that act as monocyte depleting agents. For example, the uses of Liposomal Bisphosphonates (e.g., Clodronate) has been previously disclosed in U.S. Patent Application publication US 2012/0156280, the content of which is incorporated herein by reference in its entirety.

In certain embodiments, compositions that include both: (i) an angiotensin II receptor blocker (ARB) or a compound of Formula (I); and (ii) an antigen, a vaccine, or an anti-tumor preparation, as described in greater detail herein, can be used to reduce or inhibit monocyte recruitment to lymph nodes, enhance the individual's immune response against the antigen, increase or amplify vaccine immunity, or reduce or inhibit tumor growth or metastasis. Similarly, in certain embodiments, compositions that include an angiotensin II receptor blocker (ARB) or a compound of Formula (I), when used together with separate compositions that include an antigen, a vaccine, or an anti-tumor preparation, as described in greater detail herein, can be used to reduce monocyte recruitment to lymph nodes, enhance
the individual's immune response against the antigen, amplify vaccine immunity, or inhibit tumor growth or metastasis. Similarly, in certain embodiments, compositions that include an angiotensin II receptor blocker (ARB) or a compound of Formula (I), when used together with separate compositions that include an antigen, a vaccine, or an anti-tumor preparation, as described in greater detail herein, can be used to reduce monocyte recruitment to lymph nodes, enhance the individual's immune response against the antigen, amplify vaccine immunity, or inhibit tumor growth or metastasis.

Accordingly, in certain embodiments, the present invention provides a composition comprising an ARB or a Compound of Formula (I). In certain embodiments, the present invention includes a composition comprising an antigen, a vaccine, or a tumor preparation. In certain embodiments, the present invention provides a composition comprising an ARB and an antigen, a vaccine, or a tumor preparation. In certain embodiments, the present invention provides a composition comprising (i) a Compound of Formula (I); and (ii) an antigen, a vaccine, or a tumor preparation. In certain embodiments, such compositions further comprise a pharmaceutically acceptable carrier. In certain embodiments, such compositions are pharmaceutically acceptable compositions. In certain embodiments, such compositions are administered to an individual. In certain embodiments, such compositions are used to reduce monocyte recruitment to lymph nodes, enhance the individual's immune response against the antigen, amplify vaccine immunity, or to inhibit tumor growth.

In some embodiments, these compositions also comprise one or more other monocyte migration inhibitors, such as for example one or more CCR2 inhibitor. In some other embodiments, these compositions are administered before, after, or in conjunction with other monocyte migration inhibitors, such as for example one or more CCR2 inhibitors. In certain embodiments, such compositions (comprising an ARB or a Compound of Formula (I) and optionally comprising one or more other monocyte migration inhibitor, and further optionally comprising an antigen, vaccine, or anti-tumor preparation) are used to reduce monocyte recruitment to lymph nodes, enhance the individual's immune response against the antigen, amplify vaccine immunity, or to inhibit tumor growth or metastasis. As is demonstrated in the Examples, such combination of monocyte migration inhibitors can lead to more efficacious block of monocyte migration. Accordingly, in certain embodiments, the
invention further provides a composition comprising a CCR2 inhibitor. The compositions of
the present invention may also be combined with (e.g., into a single composition), or used
with (i.e., administered before, after, simultaneous to, or in conjunction with) other
compounds (e.g., non-specific immunostimulants or receptor tyrosine kinase inhibitors).

Myeloid cells and especially CCR2+ inflammatory monocytes play an important role
in creating favorable conditions for seeding and growth of tumor metastases in the lungs, in
part by establishing the so-called metastatic niche. Compositions that comprise singularly or
in combination an angiotensin II receptor blocker (ARB), a compound of Formula (I), and/or
a CCR2 antagonist, as described in greater detail herein, can be used to inhibit monocyte
migration into tumor tissues and to suppress tumor growth and/or metastasis. Such
compositions may also comprise or be used in combination with other compounds (e.g., non-
specific immunostimulants or receptor tyrosine kinase inhibitors) to suppress tumor growth
and/or metastasis.

Angiotensin II Receptor Blockers (ARBs).

Certain embodiments of the invention relate to Angiotensin II receptor blockers (also
known as angiotensin receptor antagonists, ATi-receptor antagonists, or sartans), referred to
herein as "ARB"s. ARBs are a group of pharmaceuticals which modulate the renin-
angiotensin-aldosterone system by selectively inhibiting the effects of angiotensin II (Ang
II), a peptide hormone that plays an important role in the pathophysiology of hypertension.

ARBs antagonize the action of Ang II at the Ang II type 1 (ATi) receptor and produce their
blood pressure lowering effects by reversing the effects of Ang II, including, e.g.,
vasoconstriction, aldosterone release, ADH secretion, ACTH secretion, increased sodium
absorption by the kidney, and catecholamine release. Losartan was the first ARB to be
developed and approved by the United States Food and Drug Administration (FDA), and it
has served as the basis for the development of other ARBs, including Azilsartan,
Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, and Valsartan, each of which
is also approved by the FDA for clinical use.

Accordingly, ARBs can be used treat hypertension; however, ARBs can be
prescribed for other indications, as well. For example, Losartan can also be used to reduce
the risk of stroke in patients with hypertension and left ventricular hypertrophy and/or to
treat diabetic nephropathy in patients with type 2 diabetes, an elevated serum creatinine and proteinuria, and a history of hypertension. Candesartan can be used in the treatment of heart failure in adults with left ventricular systolic dysfunction to reduce the risk of death and/or hospitalizations due to heart failure. Irbesartan is indicated for the treatment of diabetic nephropathy in patients with type 2 diabetes, hypertension, and an elevated serum creatinine and proteinuria. Telmisartan can be used to reduce the risk myocardial infarction, stroke, or death from cardiovascular causes in patients at high risk of developing major cardiovascular events. Valsartan can be used for the treatment of heart failure and used to reduce the risk of death in patients with left ventricular failure or left ventricular dysfunction following myocardial infarction. The benefits of ARB treatment has also been tested for a variety of other diseases and disorders, including, e.g., congestive heart failure, chronic heart failure, migraine, stroke, and renal disease (Gales et al. (2010) Ann. Pharmacother. 44 (2): 360-6; Irie et al. (2012) Int J Cardiol. Published online July 16, 2012; Kobori et al. (2012) Curr Pharm Des. Published online November 21, 2012; Cancian et al. (2012) Eur J Gen Pract. Published on line September 24, 2012). Each of the ARBs described above is known by a number of trade names, the most common of which are listed in Table 1 herein.

Table 1: ARBs and Trade Names

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azilsartan</td>
<td>Edarbi®</td>
</tr>
<tr>
<td>Candesartan</td>
<td>Blopress®, Atacand®</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>Teveten®</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>Avapro®</td>
</tr>
<tr>
<td>Losartan</td>
<td>Cozaar®, Anzar®, Arbloc®, Angisartan® Hartzar®, Pharex®, Neosartan®, Hyoerthree®, Getzar®, Kenzar®, Lozaris®, Qxar®, Normoten®, Ecozar®, Lifezar®</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>Olmezar®, Olmetec®</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>Micardis®, Pritor®, Benicar®</td>
</tr>
<tr>
<td>Valsartan</td>
<td>Diovan®</td>
</tr>
</tbody>
</table>
In certain embodiments, methods described herein comprise administering an ARB, and compositions and kits described herein comprise an ARB. In certain embodiments, compositions and kits of the invention include any ARB, any combination of ARBs, or any prodrug, salt, or derivative of any ARB shown in Table 1. Accordingly, in certain embodiments, the compositions of the invention include Losartan and an antigen, vaccine, or anti-tumor preparation, where the Losartan in the composition is at a concentration sufficient to provide a dose of at least about 5 mg, at least about 10 mg, at least about 15 mg, at least about 20 mg, at least about 25 mg, at least about 30 mg, at least about 35 mg, at least about 40 mg, at least about 45 mg, at least about 50 mg, at least about 60 mg, at least about 70 mg, at least about 80 mg, of Losartan, including any range in between these values, or less than about 50 mg, or less than about 25 mg of Losartan, including any range in between these values. In certain embodiments, the Losartan in the composition is at a concentration sufficient to provide a dose of more than about 80 mg, more than about 100 mg, more than about 125 mg, more than about 150 mg, more than about 175 mg, or more than about 200 mg or Losartan, including any range in between about 5 mg and about 200 mg.

In certain embodiments, compositions of the invention can include Losartan at a concentration sufficient to provide a dose of at least about 0.5 mg/kg, at least about 0.75 mg/kg, at least about 1.0 mg/kg, at least about 1.25 mg/kg, at least about 1.5 mg/kg, at least about 1.75 mg/kg, or at least about 2.0 mg/kg of Losartan, including any range between about 0.5 mg/kg and about 1.75 mg/kg. In certain embodiments, compositions of the invention can include Losartan at a concentration sufficient to provide a dose of more than about 1.75 mg/kg, at least about 2.0 mg/kg, at least about 5 mg/kg, at least about 7 mg/kg, at least about 10 mg/kg, at least about 12 mg/kg, at least about 15 mg/kg, at least about 17 mg/kg, at least about 20 mg/kg, at least about 22 mg/kg, at least about 25 mg/kg, at least about 27 mg/kg, or at least about 30 mg/kg or Losartan, including any range in between about 1.75 mg/kg and about 30 mg/kg. In certain embodiments, the compositions of the invention can include Losartan at a concentration sufficient to provide a dose of more than about 30 mg/kg, e.g., at least about 35 mg/kg or at least about 40 mg/kg of Losartan, including any range in between about 30 mg/kg and about 40 mg/kg.
For example, the invention provides compositions that can include Azilsartan and an antigen, vaccine, or anti-tumor preparation, where the Azilsartan in the composition is at a concentration sufficient to provide a dose of at least about 10 mg, at least about 20 mg, at least about 40 mg, at least about 60 mg, at least about 70 mg, at least about 80 mg, of Losartan, including any range in between these values, or less than about 50 mg, or less than about 80 mg, including any range between these values. In certain embodiments, the Azilsartan in the composition is at a concentration sufficient to provide a dose of more than about 80 mg, more than about 100 mg, more than about 120 mg, more than about 140 mg, or more than about 160 mg, including any range in between these values.

The invention also provides compositions that can include Candesartan and an antigen, vaccine, or anti-tumor preparation, where the Candesartan in the composition is at a concentration sufficient to provide a dose of at least about 0.5 mg, at least about 1 mg, at least about 2 mg, at least about 3 mg, or less than about 4 mg, including any range between these values. In certain embodiments, the Candesartan in the composition is at a concentration sufficient to provide a dose of more than about 32 mg, more than about 40 mg, more than about 48 mg, more than about 56 mg, more than about 64 mg or more than about 84 mg, including any range in between these values. In certain embodiments, the Candesartan in the composition is at a concentration sufficient to provide a dose of less than about 32 mg, less than about 40 mg, less than about 48 mg, less than about 56 mg, less than about 64 mg or less than about 84 mg, including any range in between these values.

Certain compositions of the invention can include Eprosartan and an antigen, vaccine, or anti-tumor preparation, where the Eprosartan in the composition is at a concentration sufficient to provide a dose of at least about 50 mg, at least about 100 mg, at least about 200 mg, at least about 300 mg, at least about 400 mg, at least about 500 mg, at least about 600 mg, at least about 700 mg, at least about 800 mg, including any range between these values, or less than about 400 mg, less than about 300 mg, less than about 200 mg, or less than about 100 mg, including any range between these values. In certain embodiments, the Eprosartan in the composition is at a concentration sufficient to provide a dose of more than about 600 mg, more than about 750 mg, more than about 900 mg, more than about 1050 mg, or more than about 1200 mg, including any range in between these values.
Certain compositions of the invention can include Irbesartan and an antigen, vaccine, or anti-tumor preparation, where the Irbesartan in the composition is at a concentration sufficient to provide a dose of at least about 12.5 mg, at least about 25 mg, at least about 50 mg, at least about 75 mg, at least about 100 mg, at least about 125 mg, at least about 150 mg, at least about 175 mg, at least about 200 mg, at least about 250 mg, at least about 275 mg, or at least about 300 mg, including any range between these values, or less than about 150 mg, less than about 100 mg, less than about 50 mg, including any range between these values. In certain embodiments, the Irbesartan in the composition is at a concentration sufficient to provide a dose of more than about 300 mg, more than about 375 mg, more than about 450 mg, more than about 525 mg, or more than about 600 mg, including any range in between these values.

Alternatively, compositions of the invention can include Olmesartan and an antigen, vaccine, or anti-tumor preparation, where the Olmesartan in the composition is at a concentration sufficient to provide a dose of at least about 5 mg, at least about 10 mg, at least about 15 mg, at least about 20 mg, at least about 25 mg, at least about 30 mg, at least about 35 mg, at least about 40 mg, including any range between these values, or less than about 20 mg, less than about 15 mg, less than about 10 mg including any range between these values. In certain embodiments, the Olmesartan in the composition is at a concentration sufficient to provide a dose of more than about 40 mg, more than about 50 mg, more than about 60 mg, more than about 70 mg, or more than about 40 mg, including any range in between these values.

Compositions of the invention can include Telmisartan and an antigen, vaccine, or anti-tumor preparation, where the Telmisartan in the composition is at a concentration sufficient to provide a dose of at least about 5 mg, at least about 10 mg, at least about 15 mg, at least about 20 mg, at least about 25 mg, at least about 30 mg, at least about 35 mg, at least about 40 mg, including any range between these values or less than about 20 mg, less than about 15 mg, less than about 10 mg, including any range between these values. In certain embodiments, the Telmisartan in the composition is at a concentration sufficient to provide a dose of more than about 80 mg, more than about 100 mg, more than about 120 mg, more than about 140 mg, or more than about 160 mg, including any range in between these values.
Compositions of the invention can include Valsartan and an antigen, vaccine, or anti-tumor preparation, where the Valsartan in the composition is present at a concentration sufficient to provide a dose of at least about 5mg, at least about 10 mg, at least about 15 mg, at least about 20mg, at least about 25 mg, at least about 30 mg, at least about 35 mg, at least about 40 mg, including any range between these values, or less than about 20 mg, less than about 15 mg, less than about 10 mg, including any range between these values. In certain embodiments, the Valsartan in the composition is at a concentration sufficient to provide a dose of more than about 320 mg, more than about 400 mg, more than about 480 mg, more than about 560 mg, or more than about 640 mg, including any range in between these values.

In some embodiments, the compositions comprise a mixture of 2 or more ARBs. In some aspects, the compositions may comprise about 2 to about 8, or about 2 to about 6, or about 2 to about 4, or 1, 2, 3, 4, 5, 6, 7, 8 or more ARBs as described herein. In some embodiments, methods of the present invention comprise administering 2 or more ARBs. In some aspects the methods comprise administering about 2 to about 8, or about 2 to about 6, or about 2 to about 4, or 1, 2, 3, 4, 5, 6, 7, 8 or more ARBs as described herein. Typically, ARBs are supplied in the form of tablets for oral administration. ARBs each exhibit different pharmacokinetic properties. For example, as shown below in Table 2, the biological half-lives and the bioavailability of ARBs vary widely, with Losartan having the lowest in vivo half-life.
Table 2: Comparison of ARB Pharmacokinetics

<table>
<thead>
<tr>
<th>Drug</th>
<th>Biological Half-Life</th>
<th>Bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azilsartan</td>
<td>11 hours</td>
<td>60%</td>
</tr>
<tr>
<td>Candesartan</td>
<td>9 hours</td>
<td>15%</td>
</tr>
<tr>
<td>Eprosartan</td>
<td>5 hours</td>
<td>13%</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>11-15 hours</td>
<td>70%</td>
</tr>
<tr>
<td>Losartan</td>
<td>2 hours</td>
<td>33%</td>
</tr>
<tr>
<td>Olmesartan</td>
<td>14-16 hours</td>
<td>29%</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>24 hours</td>
<td>42-58%</td>
</tr>
<tr>
<td>Valsartan</td>
<td>6 hours</td>
<td>25%</td>
</tr>
</tbody>
</table>

Following administration (e.g., oral or otherwise), the presence and/or the levels of an ARB (or of its metabolites) can be detected in an individual's blood or urine using methods well known to those of skill in the art, including, for example, chromatographic and/or spectroscopic techniques. Details regarding such techniques are described in, *e.g.*, Nakashima et al. (1996) *Blood Press. Suppl.* 2: 62-66; Sica et al. (2005) *Clin. Pharmacokinet.* 44(8): 797-814; Lu et al. (2011) *J. Pharm. Biomed. Anal.* 54(1): 100-105; Yeung et al. (2000) *Int. J. Pharmaceut.* 204: 17-22; Chando et al. (1998) *Drug Metab.* *Dispos.* 26(5): 408-417; McCarthy et al. (1998) *J. Pharm Biomed. Anal.* 17: 671-677; Ferreiros et al. (2007) *Ther. Drug Monitoring* 29(6): 824-834; Gonzalez et al. (2002) *J. Chromatography A* 949: 49-60; and others.

**Compounds of Formula (I)-(V)**

Certain embodiments of the invention relate to compounds of any one of Formulae (I) - (V). In certain embodiments, methods described herein comprise administration of compositions comprising a compound of any one of Formulae (I)-(V), and compositions and kits described herein comprise a compound of any one of Formulae(I)-(V). Formulae (I)-(V) are described below.
**Formula (I)**

The present disclosure provides a compound of Formula (I) and compositions comprising a compound of Formula (I):

![Chemical Structure](attachment:image.png)

wherein

- $R^1$ is hydrogen or $C_{1-6}$ alkyl;
- $\equiv$ is a single bond or double bond;
- $Q^1$ is N or CH;
- $R^2$ is selected from hydrogen and $C_{1-6}$ alkyne, wherein one carbon unit of said alkyne is optionally replaced with $-O-$, $-S-$, $-SO-$, $-SO_2-$, $-NR^a-$, or $-CO-$; wherein $R^a$ is hydrogen or $C_{1-6}$ alkyl; and
- $R^3$ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with $C_{1-6}$ alkyl;

or pharmaceutically acceptable salts thereof.

In some embodiments of Formula (I), $R^1$ is hydrogen. In some embodiments, $R^1$ is $C_{1-6}$ alkyl. In some embodiments, $R^1$ is methyl or ethyl. In some embodiments, $R^1$ is methyl.

In some embodiments of Formula (I), $\equiv$ is a single bond. In some embodiments, $\equiv$ is a double bond.

In some embodiments of Formula (I), $Q^1$ is N. In some embodiments, $Q^1$ is CH.

In some embodiments of Formula (I), $R^2$ is hydrogen. In some embodiments, $R^2$ is a $C_{1-6}$ alkyne, such as $C_1$ alkyne, $C_2$ alkyne, $C_3$ alkyne, $C_4$ alkyne, $C_5$ alkyne, or $C_6$ alkyne. In some embodiments, $R^2$ is a $C_1$ alkyne. In some embodiments, $R^2$ is a $C_{1-6}$ alkyne, wherein one carbon unit of said alkyne is replaced with $-O-$, $-S-$, $-SO-$, $-SO_2-$, $-NR^a-$, or $-CO-$; wherein $R^a$ is hydrogen or $C_{1-6}$ alkyl. In some embodiments, $R^2$ is a $C_{1-6}$ alkyne, wherein one carbon unit of said alkyne is replaced with $-O-$, $-S-$, $-SO-$, or $-SO_2-$.

In some embodiments, $R^2$ is a $C_{1-6}$ alkyne, wherein one carbon unit of said alkyne is
replaced with -NRα-; wherein Rα is hydrogen or Ci₆ alkyl. In some embodiments, R² is Ci₆ alkylene, wherein one carbon unit of said alkylene is replaced with -CO-.

In some embodiments of Formula (I), R³ is hydrogen. In some embodiments, R³ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Ci₆ alkyl. In some embodiments, R³ is an optionally substituted 5-membered heteroaryl ring containing one, two, three, or four heteroatoms. In certain instances, the R³ heteroaryl ring contains one heteroatom. In certain instances, the R³ heteroaryl ring contains two heteroatoms. In certain instances, the R³ heteroaryl ring contains three heteroatoms. In certain instances, the R³ heteroaryl ring contains four heteroatoms. In certain instances, the R³ heteroaryl ring contains at least one heteroatom selected from nitrogen, sulfur, and oxygen. In certain instances, the R³ heteroaryl ring contains two heteroatoms selected from nitrogen, sulfur, and oxygen. In certain instances, the R³ heteroaryl ring contains two nitrogen heteroatoms. In certain instances, the R³ heteroaryl ring contains carbon, nitrogen, and sulfur ring members. In certain instances, the R³ heteroaryl ring contains carbon and nitrogen ring members. In certain instances, the R³ heteroaryl ring contains carbon, nitrogen, and oxygen ring members.

In certain instances, the R³ heteroaryl ring is furanyl, thiophenyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, thiadiazolyl, oxadiazolyl, triazolyl, or tetrazolyl, each optionally substituted with Ci₆ alkyl, as described for the R³ heteroaryl ring. In certain instances, the R³ heteroaryl ring is selected from the following:

![Heteroaryl ring structures](image)

each optionally substituted with Ci₆ alkyl, as described for the R³ heteroaryl ring. In certain instances, the R³ heteroaryl ring is

**Formula (II)**

The present disclosure provides a compound of Formula (II) and compositions comprising a compound of Formula (II):
wherein

R₁ is hydrogen or C₆alkyl;

== is a single bond or double bond;

Q₁ is N or CH; and

R₄ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C₆alkyl;

or pharmaceutically acceptable salts thereof.

In some embodiments of Formula (II), R₁ is hydrogen. In some embodiments, R₁ is C₆alkyl. In some embodiments, R₁ is methyl or ethyl. In some embodiments, R₁ is methyl.

In some embodiments of Formula (II), == is a single bond. In some embodiments, == is a double bond.

In some embodiments of Formula (II), Q₁ is N. In some embodiments, Q₁ is CH.

In some embodiments of Formula (II), R₄ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C₆alkyl. In some embodiments, R₄ is an optionally substituted 5-membered heteroaryl ring containing one, two, three, or four heteroatoms. In certain instances, the R₄ heteroaryl ring contains one heteroatom. In certain instances, the R₄ heteroaryl ring contains two heteroatoms. In certain instances, the R₄ heteroaryl ring contains three heteroatoms. In certain instances, the R₄ heteroaryl ring contains four heteroatoms. In certain instances, the R₄ heteroaryl ring contains at least one heteroatom selected from nitrogen, sulfur, and oxygen. In certain instances, the R₄ heteroaryl ring contains two heteroatoms selected from nitrogen, sulfur, and oxygen. In certain instances, the R₄ heteroaryl ring contains two nitrogen heteroatoms. In certain instances, the R₄ heteroaryl ring contains carbon, nitrogen, and sulfur ring members. In certain instances, the R₄ heteroaryl ring contains carbon and nitrogen ring members. In certain instances, the R₄ heteroaryl ring contains carbon, nitrogen, and oxygen ring members.
In certain instances, the $R^4$ heteroaryl ring is furanyl, thiophenyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, thiadiazolyl, oxadiazolyl, triazolyl, or tetrazolyl, each optionally substituted with $C_{1-6}$ alkyl, as described for the $R^4$ heteroaryl ring. In certain instances, the $R^4$ heteroaryl ring is selected from the following:

![Chemical structures]

each optionally substituted with $C_{1-6}$ alkyl, as described for the $R^3$ heteroaryl ring. In certain instances, the $R^4$ heteroaryl ring is

or

**Formula (III)**

The present disclosure provides a compound of Formula (III) and compositions comprising a compound of Formula (III):

![Chemical structure]

wherein

\[\equiv\] is a single bond or double bond;

$Q^1$ is N or CH; and

$R^4$ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with $C_{1-6}$ alkyl;
or pharmaceutically acceptable salts thereof.

In some embodiments of Formula (III), \[\equiv\] is a single bond. In some embodiments, \[\equiv\] is a double bond.

In some embodiments of Formula (III), $Q^1$ is N. In some embodiments, $Q^1$ is CH.

In some embodiments of Formula (III), $R^4$ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with $C_{1-6}$ alkyl. In some embodiments, $R^4$ is an optionally substituted 5-membered heteroaryl ring containing one,
two, three, or four heteroatoms. In certain instances, the R⁴ heteroaryl ring contains one heteroatom. In certain instances, the R⁴ heteroaryl ring contains two heteroatoms. In certain instances, the R⁴ heteroaryl ring contains three heteroatoms. In certain instances, the R⁴ heteroaryl ring contains four heteroatoms. In certain instances, the R⁴ heteroaryl ring contains at least one heteroatom selected from nitrogen, sulfur, and oxygen. In certain instances, the R⁴ heteroaryl ring contains two heteroatoms selected from nitrogen, sulfur, and oxygen. In certain instances, the R⁴ heteroaryl ring contains two nitrogen heteroatoms. In certain instances, the R⁴ heteroaryl ring contains carbon, nitrogen, and sulfur ring members. In certain instances, the R⁴ heteroaryl ring contains carbon and nitrogen ring members. In certain instances, the R⁴ heteroaryl ring contains carbon, nitrogen, and oxygen ring members.

In certain instances, the R⁴ heteroaryl ring is furanyl, thiophenyl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, thiadiazolyl, oxadiazolyl, triazolyl, or tetrazolyl, each optionally substituted with C₆₆ alkyl, as described for the R⁴ heteroaryl ring. In certain instances, the R⁴ heteroaryl ring is selected from the following:

![Chemical Structures]

each optionally substituted with C₆₆ alkyl, as described for the R³ heteroaryl ring. In certain instances, the R⁴ heteroaryl ring is

**Formula (IV)**

The present disclosure provides a compound of Formula (IV) and compositions comprising a compound of Formula (IV):

![Chemical Structure]

wherein

R¹ is hydrogen or C₆₆ alkyl;
is a single bond or double bond;

Q is N or CH; and

R is selected from

or pharmaceutically acceptable salts thereof.

In some embodiments of Formula (IV), R is hydrogen. In some embodiments, R is C₆H₅ alkyl. In some embodiments, R is methyl or ethyl. In some embodiments, R is methyl.

In some embodiments of Formula (IV), is a single bond. In some embodiments, is a double bond.

In some embodiments of Formula (IV), Q is N. In some embodiments, Q is CH.

In some embodiments of Formula (IV), R is

In some embodiments, R is

**Formula (V)**

The present disclosure provides a compound of Formula (V) and compositions comprising a compound of Formula (V):

wherein

is a single bond or double bond;

Q is N or CH; and

R is selected from

or pharmaceutically acceptable salts thereof.
In some embodiments of Formula (V), \( \equiv \equiv \) is a single bond. In some embodiments, \( \equiv \equiv \) is a double bond.

In some embodiments of Formula (V), \( Q^1 \) is N. In some embodiments, \( Q^1 \) is CH.

In some embodiments of Formula (V), \( R^4 \) is :\[ \text{Diagram} \]

In some embodiments, \( R^4 \) is :\[ \text{Diagram} \]

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination. All combinations of the embodiments pertaining to the chemical groups represented by the variables are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace compounds that are stable compounds (i.e., compounds that can be isolated, characterized, and tested for biological activity). In addition, all subcombinations of the chemical groups listed in the embodiments describing such variables are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination of chemical groups was individually and explicitly disclosed herein.

Particular compounds of interest are shown below:

Ondansetron;

9-methyl-3-((2-methyl-1H-imidazol-1-yl)methyl)-2,3-dihydro-1H-carbazol-4(9H)-one
Alosetron can be used in the management of severe diarrhea-predominant irritable bowel syndrome (IBS). Ondansetron can be prescribed to treat and/or prevent chemotherapy-induced nausea and vomiting (CINV). Ondansetron has been indicated in the prevention and treatment of radiation-induced nausea and vomiting (RINV), and post-operative nausea and vomiting (PONV). The benefits of Ondansetron treatment have also been tested for a variety of other diseases and disorders, including, e.g., motion sickness (Levine et al. (2000) Aviat Space Environ Med. 71: 1111-1114; Muth et al. (2007) Aviat Space Environ Med. 78: 686-92); lesional vestibular disorder (European Patent Application No. 2432467 A1 and US Patent Application Publication 2012/0064094); anti-psychotic induced tardive dyskinesia in people with schizophrenia (Zullino et al. (2001) Am. J. Psychiatry 158: 657-8 and Sirota et al. (2000) Am. J. Psychiatry 157: 287-289); and schizophrenia (Zhang et al. (2006) Schiz Res 88: 102-110). Other medical conditions that may be treated using ondansetron include, e.g., gastroenteritis, pediatric gastroenteritis, opioid-induced nausea, nausea and vomiting of pregnancy, and obsessive-compulsive disorder (Broocks et al. (1998) Psychiatry Res. 79: 11-20).

Ondansetron and Alosetron are each known by a number of trade names, the most common of which are listed in Table 3 herein.
Table 3: Trade Names

<table>
<thead>
<tr>
<th>Trade Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alosetron</td>
</tr>
<tr>
<td>Ondansetron</td>
</tr>
</tbody>
</table>

Accordingly, in certain embodiments, the compositions of the invention include Ondansetron and an antigen, vaccine, or anti-tumor preparation. In certain embodiments, the composition is administered orally or intravenously. In certain embodiments, Ondansetron in the orally or intravenously administered composition is at a concentration sufficient to provide a dose of at least about 0.5 mg, at least about 1 mg, at least about 2 mg, at least about 3 mg, at least about 4 mg, at least about 5 mg, at least about 6 mg, at least about 7 mg, at least about 8 mg, at least about 10 mg, or less than about 12 mg Ondansetron, including any range in between these values. In certain embodiments, the Ondansetron in the orally administered composition is at a concentration sufficient to provide a dose of more than about 24 mg, more than about 26 mg, more than about 28 mg, more than about 30 mg, more than about 32 mg, or more than about 34 mg Ondansetron, including any range in between these values.

In certain embodiments, compositions of the invention are administered intravenously. Compositions for intravenous administration can include Ondansetron at a concentration sufficient to provide a dose of at least about 0.01 mg/kg/day, at least about 0.05 mg/kg/day, at least about 0.1 mg/kg/day, at least about 0.15 mg/kg/day, at least about 0.2 mg/kg/day, at least about 0.3 mg/kg/day, at least about 0.4 mg/kg/day, at least about 0.5 mg/kg/day, at least about 0.75 mg/kg/day, at least about 1.0 mg/kg/day, at least about 1.5 mg/kg/day, at least about 2.0 mg/kg/day, at least about 2.5 mg/kg/day, at least about 3.0 mg/kg/day, at least about 3.5 mg/kg/day, at least about 4.0 mg/kg/day, at least about 4.5 mg/kg/day, at least about 5.0 mg/kg/day, at least about 5.5 mg/kg/day, at least about 6.0 mg/kg/day, at least about 6.5 mg/kg/day, at least about 7.0 mg/kg/day, at least about 7.5 mg/kg/day, at least about 8.0 mg/kg/day, or less than about 8.5 mg/kg/day of Ondansetron, including any range between these values.
For example, the invention provides compositions that can include Alosetron and an antigen, vaccine, or anti-tumor preparation, where the Alosetron in the composition is at a concentration sufficient to provide a dose of at least about 0.1 mg, at least about 0.2 mg, at least about 0.3 mg, at least about 0.4 mg, or less than about 0.5 mg Alosetron, including any range between these values. In certain embodiments, the Alosetron in the composition is at a concentration sufficient to provide a dose of more than about 2 mg, more than about 3 mg, more than about 4 mg, more than about 5 mg, or more than about 6 mg Alosetron, including any range in between these values.

Ondansetron and Alosetron are supplied in the form of tablets or solutions for oral administration. Ondansetron is also supplied in solution form for parenteral administration. Ondansetron and Alosetron each exhibit different pharmacokinetic properties. For example, the biological half-life of Alosetron is about 1.5 to about 1.7 hours, and the biological half-life of Ondansetron is about 3.9 hours.

Following administration (e.g., oral, intramuscular, subcutaneous, or otherwise), the presence and/or the levels of a compound of Formula (I) (or of its metabolites) can be detected in an individual’s blood or urine using methods well known to those of skill in the art, including, for example, chromatographic and/or spectroscopic techniques. Details regarding such techniques are described in, e.g., Somers et al. (2007) Xenobiotica 37: 855-869; Koch et al. (2002) Br J Clin Pharmacol. 53: 238-242; Xu et al. (2000) J Mass Spectrom 35: 1329-1334; Roila et al. (1995) Clin Pharmacokinet 29: 95-103; and others.

**CCR2 antagonists**

Certain embodiments of the invention relate to CCR2 antagonists and compositions comprising CCR2 antagonists (e.g., as are described in US patent application publication US 2012/0156280, incorporated herein by reference in its entirety). The major chemokine regulating monocyte recruitment is MCP-1 (CCL2), which signals primarily via activation of the receptor CCR2 expressed principally on inflammatory monocytes. Several specific small molecule inhibitors of CCR2 have been developed for treatment of inflammatory diseases such as rheumatoid arthritis, asthma, and atherosclerosis. The present disclosure demonstrates the use of several exemplary small molecule CCR2 inhibitors for use in methods for suppressing tumor growth or metastasis in an individual with cancer, and
methods for synergistically suppressing monocyte migration in conjunction with other monocyte migration inhibitors, however it is explicitly considered that virtually any CCR2 inhibitor and/or antagonist that demonstrates monocyte migration inhibiting activity as described herein, is suitable for use according to the present disclosure. Moreover, such CCR2 antagonists may be used alone or in combination with other compositions of the present invention (e.g., one or more other MMI, TKIs, non-specific immunostimulants, antigens, vaccines, anti-tumor preparations, common vaccine adjuvants, etc, alone or in combination) for various uses, e.g., enhancing an immune response, inhibiting or reducing monocyte migration (e.g., migration to a lymphnode such as a draining lymph node), amplifying vaccine immunity, or suppressing tumor growth or metastasis.

Applicants incorporate specifically herein by reference the disclosure of Higgins et al. (2007) (Chemokine Biology-Basic Research an Clinical Application, Vol. II, Birkhauser Verlag Basel Switzerland, pg. 115-123) and the disclosure of Mirzadegan et al. (2000)(The Journal of Biological Chemistry, Vol. 275, No.33, Aug, pg. 25562-25571) in their entirety. While not intending to be limited in any way by this exemplary set of CCR2 molecules, Table 4 herein presents a number of CCR2 antagonists that are considered to be suitable for use according to the present invention.

<table>
<thead>
<tr>
<th>Table 4: CCR2 antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Roche/Genentech</td>
</tr>
</tbody>
</table>

CCR2 IC(50) - 89 nM bind, 210 ω i taxis
<table>
<thead>
<tr>
<th>Company</th>
<th>CCR2 Antagonists</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millennium/Pfizer</td>
<td><img src="" alt="Benzamidazoles" /></td>
</tr>
<tr>
<td>SmithKline</td>
<td><img src="" alt="SB-380732" /> 50 nM bind</td>
</tr>
<tr>
<td>AstaZeneca</td>
<td>AZD-6942 29 nM bind 69 nM toxicity</td>
</tr>
<tr>
<td>Merck</td>
<td><img src="" alt="41" /> 41 nM bind 59 nM toxicity</td>
</tr>
<tr>
<td>Teijin/BMS</td>
<td><img src="" alt="3-Aminopyrrolidines" /> 3 nM</td>
</tr>
<tr>
<td>Telik</td>
<td><img src="" alt="" /></td>
</tr>
</tbody>
</table>
In addition, many other CCR2 inhibitors and/or CCR2 antagonists in development (e.g., but not limited to, RS-21825, RS-29634, and RS-136270, disclosed in Mirzadegan et al, supra and PF-04 17890) are considered to be suitable for use according to the present disclosure.

Particular compounds of interest from the list above are RSI 02895 (e.g., from Tocris, Catalog number 2089) and RS504393 (Roche/Iconix), each of which are included in Table 4, herein.

Accordingly, in certain embodiments, the compositions of the invention comprise RSI 02895 or RS504393 singularly, or in combination with one or more of the other monocyte migration inhibitors disclosed herein. In certain embodiments, the composition is administered orally. In certain embodiments, RSI 02895 or RS504393 in the orally administered composition is at a concentration sufficient to provide a dose of at least about 0.5 mg, at least about 1 mg, at least about 2 mg, at least about 3 mg, at least about 4 mg, at least about 5 mg, at least about 6 mg, at least about 7 mg, at least about 8 mg, at least about 10 mg, or less than about 12 mg of RSI 02895 or RS504393, including any range in between...
these values. In certain embodiments, the RSI 02895 or RS504393 in the orally administered composition is at a concentration sufficient to provide a dose of more than about 24mg, more than about 26 mg, more than about 28 mg, more than about 30 mg, more than about 32 mg, or more than about 34 mg of RSI 02895 or RS504393, including any range in between these values.

In certain embodiments, compositions of the invention are administered intravenously. Compositions for intravenous administration can include RSI 02895 or RS504393 at a concentration sufficient to provide a dose of at least about 0.5 mg/kg/day, at least about 0.75 mg/kg/day, at least about 1.0 mg/kg/day, at least about 1.5 mg/kg/day, at least about 2.0 mg/kg/day, at least about 2.5 mg/kg/day, at least about 3.0 mg/kg/day, at least about 3.5 mg/kg/day, at least about 4.0 mg/kg/day, at least about 4.5 mg/kg/day, at least about 5.0 mg/kg/day, at least about 5.5 mg/kg/day, at least about 6.0 mg/kg/day, at least about 6.5 mg/kg/day, at least about 7.0 mg/kg/day, at least about 7.5 mg/kg/day, at least about 8.0 mg/kg/day, or less than about 8.5 mg/kg/day of RS102895 or RS504393, including any range between these values.

For example, the invention provides compositions that can include RSI 02895 or RS504393 and an antigen, vaccine, or anti-tumor preparation, where the RSI 02895 or RS504393 in the composition is at a concentration sufficient to provide a dose of at least about 0.1 mg, at least about 0.2 mg, at least about 0.3 mg, at least about 0.4 mg, or less than about 0.5 mg RS102895 or RS504393, including any range between these values. In certain embodiments, the RSI 02895 or RS504393 in the composition is at a concentration sufficient to provide a dose of more than about 2 mg, more than about 3 mg, more than about 4 mg, more than about 5 mg, or more than about 6 mg RS102895 or RS504393, including any range in between these values.

RSI 02895 and RS504393 for use according to the present disclosure may be supplied, e.g., in the form of tablets or solutions for oral administration. RS102895 and RS504393 may also be supplied, e.g., in solution form for parenteral administration. RSI 02895 or RS504393 may exhibit different pharmacokinetic properties.

Following administration (e.g., oral, intramuscular, subcutaneous, or otherwise), the presence and/or the levels of a CCR2 inhibitor (or of its metabolites) can be detected in an...
individual's blood or urine using methods well known to those of skill in the art, including, for example, chromatographic and/or spectroscopic techniques. Details regarding such techniques are described in, e.g., Somers et al. (2007) Xenobiotica 37: 855-869; Koch et al. (2002) Br J Clin Pharmacol. 53: 238-242; Xu et al. (2000) J Mass Spectrom 35: 1329-1334; Roila et al. (1995) Clin Pharmacokinet 29: 95-103; and others.

**Non-specific Immunostimulants**

Certain embodiments of the invention relate to non-specific immunostimulants and compositions comprising non-specific immunostimulants. Such non-specific immunostimulants are well known to one skilled in the art, and all such non-specific immunostimulants are suitable for use according to the present disclosure. In certain embodiments, such non-specific immunostimulants include, but are not limited, to cationic lipid DNA complexes (CLDC), CpG-oligonucleotides, poly I:C, LPS, alpha-galactosylceramide, and the like.

In certain embodiments, such non-specific immunostimulants are used in combination with one of more of the compounds or compositions of the present invention (e.g., one or more other MMI, TKIs, antigens, vaccines, anti-tumor preparations, common vaccine adjuvants, etc, alone or in combination) for the various uses disclosed herein, e.g., enhancing an immune response, inhibiting or reducing monocyte migration (e.g., migration to a lymphnode such as a draining lymph node), amplifying vaccine immunity, or suppressing tumor growth or metastasis.

**Combinations of monocyte migration inhibitors**

The monocyte migration inhibitors disclosed herein can be divided into three different classes based upon their known mechanisms of action. For example, in Class I there are several angiotensin receptor blocking drugs (ARBs) such as losartan, irbesartan and telmisartan that each exert MMI activity. In Class II, there is an anti-nausea drug ondansetron (a serotonin receptor antagonist) that also exerts MMI activity. And in Class III, there are drugs which were specifically designed as MMIs, and function as pure antagonists of the CCR2 receptor on monocytes. Two examples (RS102895, and RS504393) of class III drugs are listed here, but there are many others in this same family that share a mechanism of
activity (competitive antagonists for CCR2) all of which are suitable for use in the present invention.

As is discussed further throughout this specification, monocyte migration inhibitor (MMI) drugs may be combined together or used in conjunction with one another for use in the methods disclosed in the present specification. In some embodiments, two or more MMI are administered to an individual according to the present disclosure to inhibit monocyte migration, to reduce or inhibit monocyte recruitment to lymph nodes, enhance the individual's immune response against the antigen, increase or amplify vaccine immunity, or reduce or inhibit tumor growth or metastasis. In certain embodiments, an MMI from one class is administered to an individual in combination with another MMI from a different class. In certain embodiments, an MMI from one class is administered to an individual in conjunction with another MMI from a different class. In certain embodiments, combination of MMIs in this way leads to additive or synergistic inhibition of monocyte migration.

In certain embodiments, a Class I MMI and a Class II MMI; or a Class I MMI and a Class III MMI, or a Class II MMI and a Class III MMI, or a Class I MMI, a Class II MMI, and a Class III MMI are used in combination with one another to inhibit the migration of monocytes according to any one of the methods disclosed herein. In certain embodiments, a Class I MMI and a Class II MMI; or a Class I MMI and a Class III MMI, or a Class II MMI and a Class III MMI, or a Class I MMI, a Class II MMI, and a Class III MMI are used in conjunction with one another to inhibit the migration of monocytes according to any one of the methods disclosed herein.

In particular embodiments, ondansetron is combined with (or administered in conjunction with) irbesartan, telmisartan, or losartan to inhibit monocyte migration, to reduce or inhibit monocyte recruitment to lymph nodes, to enhance the individual's immune response against the antigen, to increase or amplify vaccine immunity, or to reduce or inhibit tumor growth or metastasis, according to any one of the methods disclosed herein. In another particular embodiment, RSI02895 or RS504393 is combined with (or administered in conjunction with) irbesartan, telmisartan, or losartan to inhibit monocyte migration, to reduce or inhibit monocyte recruitment to lymph nodes, to enhance the individual's immune response against the antigen, to increase or amplify vaccine immunity, or to reduce or inhibit
tumor growth or metastasis, according to any one of the methods disclosed herein. Such combinations may in some instances result in additive increases in the monocyte migration inhibition; while in other instances, such combinations resulted in synergistic increases in monocyte migration inhibitions. Many other combinations are MMIs are contemplated, some of which are discussed in further embodiments.

In certain embodiments, the present invention provides a composition comprising any one of these combinations of MMIs (e.g., (i) Ondansetron, RS102895 or RS504393, combined with (ii) irbesartan, telmisartan, or losartan); wherein the composition optionally further comprises an antigen or a vaccine. In certain embodiments, the present invention provides a composition comprising any combination of MMIs as disclosed herein, wherein the composition optionally further comprises an antigen or a vaccine. In particular embodiments, the present invention provides a composition comprising any one of the combinations described in Table 5 herein, wherein the composition optionally further comprises an antigen or a vaccine.

In certain embodiments, the MMIs disclosed herein are also used in combination with (e.g., in conjunction with) anti-tumor or anti-cancer preparations such as are described herein, for example, receptor tyrosine kinase inhibitors (e.g., sunitinib). Such combinations can result in additive or synergistic increases in the efficiency of the MMI cancer treatment, e.g. synergistic increases in the suppression of tumor growth or metastasis.

In certain embodiments, the MMIs disclosed herein are also used in combination with (e.g., in conjunction with) non-specific immunostimulants (e.g., CLDC). Such combinations can result in the suppression of tumor growth or metastasis.

**Antigens and Vaccines**

In certain embodiments, compositions and kits of the invention include an antigen, and certain methods of the invention comprise administering an antigen. In certain embodiments, the antigen present in the compositions provided by the invention can be any material or substance that can induce an immune response (i.e., cellular and/or humoral immune response) by the immune system of a human or animal. For example, the antigen can be a polypeptide of interest derived from an infectious agent, e.g., a bacterium, a virus, a fungus, a protozoan, a parasite, or a prion. The antigen can be a whole microbe or a mixture...
thereof. The compositions can include a live whole infectious agent. In certain embodiments, the compositions can include a killed or inactivated (attenuated) infectious agent.

In certain embodiments, the antigen comprises, e.g., a polypeptide, nucleic acid, polysaccharide, a fatty acid or the like, derived from an infectious agent. In certain embodiments, the antigen can be a subunit or fragment of a polypeptide, or a fragment of a nucleic acid or polysaccharide derived from an infectious agent. In certain embodiments, the antigen is a recombinant polypeptide produced in a heterologous expression system, e.g., a recombinant protein derived from an infectious agent that was expressed in and purified from cells of another organism. However, an antigen can also be a recombinant nucleic acid construct which encodes a polypeptide antigen of interest (e.g., an expression construct). The antigen can comprise a viral subunit, a virus-like particle, a capsular (poly) saccharide; a bacterial outer membrane bleb formation containing one or more of bacterial outer membrane proteins, a phospholipid, a lipopolysaccharide, or a polysaccharide.

In certain embodiments, the antigen can be a naturally occurring substance. In certain embodiments, the antigen comprises or is derived from an allergen, e.g., pollen. In certain embodiments, the antigen comprises or is derived from a toxin. In certain embodiments, the antigen comprises or is derived from an addictive substance, including, without limitation, nicotine, caffeine, alcohol, and the like. In certain embodiments, the antigen can be a non-naturally occurring (i.e., synthetic) substance, e.g., a synthetic peptide, a synthetic polysaccharide, or a synthetic polymer.

In certain embodiments, the antigen can be provided in a vaccine, e.g., any vaccine known in the art. The vaccine can be a nucleic acid construct (e.g., a DNA vaccine). The vaccine can be a viral vector vaccine, which uses live viruses to carry DNA into an individual's cells. The DNA contained in the viral vaccine encodes antigen(s) that, once expressed in the infected cells, elicit an immune response. Alternatively, the vaccine can be a subunit vaccine, e.g., a specific protein from a virus. The vaccine can be a dendritic cell vaccine, in which an individual's dendritic cells are cultured with an antigen and then re-injected into the individual to stimulate an immune response. In certain embodiments, the vaccine can be a monovalent vaccine, i.e., containing a single antigen. In certain
embodiments, the vaccine containing the antigen is a polyvalent or multivalent vaccine, i.e., containing more than one antigen.

**Adjuvants**

Immunologic adjuvants are added to vaccines to stimulate the immune system's response to the target antigen, but do not in themselves confer immunity. Such adjuvants are well known in the art.

In certain embodiments, the present invention provides for administering a vaccine (e.g., a vaccine comprising an antigen) in combination with an adjuvant. In some embodiments, an adjuvant is mixed with a compound or composition of the present invention, and the mixture is administered to an individual. In some embodiments, an adjuvant is administered to an individual in combination with a compound or composition of the present invention (e.g., the adjuvant is administered to the individual before, after, or simultaneous with the compound or composition of the present invention). In some particular embodiments, an adjuvant is administered in conjunction with a compound or composition of the present invention.

Although immunological adjuvants have traditionally been viewed as substances that aid the immune response to antigen, adjuvants have also evolved as substances that can aid in stabilizing formulations of antigens, especially for vaccines administered for animal health.

In some embodiments, the present invention provides for an adjuvant that is a monocyte depleting agent. Such agents are well known in the art may be further described as comprising a bisphosphonate drug, such as clodronate, zoledronate, pamidronate, etidronate, or any other type of drug that is capable of depleting monocytes, and that when provided with a vaccine, provides for an enhanced immune response in an individual greater than the observed immune response in the individual given the vaccine preparation without the monocyte depleting agent. In some embodiments, the bis-phosphonate drug is a liposomal conjugated agent, such as liposomal clodronate.

In certain embodiments, the vaccine further comprises one or more adjuvant, such as the adjuvants commonly known to the skilled artisan.
Adjuvants can act in various ways in presenting an antigen to the immune system. Adjuvants can act as a depot for the antigen presenting the antigen over a long period of time, thus maximizing the immune response before the body clears the antigen. Examples of depot type adjuvants are oil emulsions. Adjuvants can also act as an irritant which causes the body to recruit and amplify immune response. A tetanus, diphtheria, and pertussis vaccine, for example, contains minute quantities of toxins produced by each of the target bacteria, but also contains some aluminum hydroxide. Such aluminum salts are common adjuvants in vaccines sold in the United States and have been used in vaccines for over 70 years. The body's immune system develops an antitoxin to the bacteria's toxins, not to the aluminum, but would not respond enough without the help of the aluminum adjuvant.

In certain embodiments, the vaccine further comprises one or more adjuvant, such as the adjuvants commonly known to the skilled artisan. By way of example, but not to be limited in any way, such common adjuvants include alum, other compounds of aluminum, Bacillus of Calmette and Guerin (BCG), TiterMax® adjuvant, Ribi®, Freund's Complete Adjuvant (FCA) and a new adjuvant disclosed by the United States Department of Agriculture's (USDA) National Wildlife Research Center on their web site at aphis.usda.gov/ws/nwrc/pzp.htm based on Johne's antigen. Alum is generally considered to be any salt of aluminum, in particular, the salts of inorganic acids. Hydroxide and phosphate salts are particularly useful as adjuvants. A suitable alum adjuvant is sold under the trade name, Imject® Alum (Pierce Chemical Company) that consists of an aqueous solution of aluminum hydroxide (45 mg/ml) and magnesium hydroxide (40 mg/ml) plus inactive stabilizers. Alum is a particularly advantageous adjuvant since it already has regulatory approval and it is widely accepted in the art.

**Anti-Tumor Preparations**

The anti-tumor preparation can be any anti-tumor preparation known and used in the art. For example, certain anti-tumor preparations inhibit the synthesis of new DNA strands, thus preventing tumor cells from replicating. In certain embodiments, the anti-tumor preparation can be an antimetabolite, such as 5-fluorouracil, methotrexate, capecitabine, Alimta, gemcitabine, etc. In certain embodiments, the anti-tumor preparation can be a
platinum-based agent, such as cisplatin, carboplatin, oxaliplatin, and the like, which cross-link DNA and inhibit DNA repair in tumor cells. In certain embodiments, the anti-tumor preparation can be an alkylating agent, such as cyclophosphamide, carmustine (BCNU), methyl-CCNU, or piposulfan. In certain embodiments, the anti-tumor agent can be a tyrosine kinase inhibitor, such as gefitinib (Iressa®), imatinib (Gleevec®), lapatinib, sunitinib (Sutent®), GW2580, or Tarceva. In certain embodiments, the anti-tumor preparation can be an anthracycline, such as actinomycin, doxil, doxorubicin (adriamycin), epirubicin, or mitoxantrone. In certain embodiments, the anti-tumor preparation can be a topoisomerase inhibitor, such as camptothecin, irinotecan, topotecan, etoposide, amsacrine, etoposide phosphate, or teniposide.

Other anti-tumor preparations interfere with microtubule assembly or disassembly, thus interrupting tumor cell division. In certain embodiments, the anti-tumor preparation can be a vinca alkaloid, such as vinblastine, vincristine, vindesine, VP-16, or vinorelbine (Navelbine®). Other anti-tumor agents that exhibit anti-microtubule activity include colchicine, taxanes and taxane derivatives. Additional anti-tumor preparations include proteasome inhibitors (e.g., bortezomib (Velcade)); anti-angiogenesis agents; and therapeutic antibodies (e.g., anti-VEGF antibody/Avastin®/bevacizumab, anti-HER2 antibody/Herceptin®/trastuzumab, Erbitux®/cetuximab, Campath/Alemtuzumab, Myelotarg/gemtuzumab tiuxetan, Rituxan/rituximab, and Bexxar/tositumomab). In certain embodiments, two or more anti-tumor preparations can be administered in combination with (e.g., in conjunction with) one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combinations thereof.

**Pharmacologically Acceptable Compositions and Formulations**

The various compounds, for example the MMIs (e.g., ARBs, CCR2 antagonists, the Compounds of Formulae (I)-(V)), the antigens, vaccines, and anti-tumor compositions described herein may be present in various compositions or formulations, including those suitable for administration to an individual (e.g., pharmaceutical compositions). The compositions of the invention can be incorporated into a variety of compositions or formulations for therapeutic administration by combination with appropriate
pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Pharmaceutical compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The carrier or diluent is selected so as not to affect the biological activity of the combination. Examples of such carriers or diluents are distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation can include other carriers, adjuvants, or non-toxic, nontherapeutic, non-immunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

The composition can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well-known compounds that enhance the in vivo stability of the polypeptide, or otherwise enhance its pharmacological properties (e.g., increase the half-life of the polypeptide, reduce its toxicity, and enhance solubility or uptake). Examples of such modifications or complexing agents include sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their in vivo attributes. Such molecules include, for example, carbohydrates, polyamines, amino acids, other peptides, ions (e.g., sodium, potassium, calcium, magnesium, manganese), and lipids.

In certain embodiments, the composition comprises a Class I MMI e.g., an angiotensin II receptor blocker such as, for example, but not to be limited, Azilsartan, Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, and Valsartan. In certain embodiments, the composition comprises a Class II MMI, e.g., a serotonin receptor antagonist such as, for example, but not to be limited, a compound of Formula (I):

\[
\text{Formula (I):}
\]
wherein

R₁ is hydrogen or Cᵢ₋₆ alkyl;

Q₁ is a single bond or double bond;

R₂ is selected from hydrogen and Cᵢ₋₆ alkyne, wherein one carbon unit of said alkyne is optionally replaced with -O-, -S-, -SO-, -SO²-, -NRₐ-, or -CO-; wherein Rₐ is hydrogen or Cᵢ₋₆ alkyl; and

R₃ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Cᵢ₋₆ alkyl;

or pharmaceutically acceptable salts thereof; e.g., a Compound of Formula II, a Compound of Formula III, a Compound of Formula IV, or a Compound of Formula V. In certain embodiments, the composition comprises Ondansetron. In certain embodiments, the composition comprises a Class III MMI, e.g., a CCR2 inhibitors such as, but not to be limited, RS102895, and RS504393 and related compounds e.g., disclosed in Higgins et al. (2007) (Chemokine Biology-Basic Research an Clinical Application, Vol. II, Birkhauser Verlag Basel Switzerland, pg. 115-123), incorporated herein by reference in its entirety, and as disclosed in Mirzadegan et al. (2000)(The Journal of Biological Chemistry, Vol. 275, No.33, Aug, pg. 25562-25571), incorporated herein by reference in its entirety. In certain embodiments, a composition such as the compositions listed herein further comprises an antigen, a vaccine, or an anti-tumor preparation. In still further embodiments, the composition further comprises a TKI or a non-specific immunostimulant.

In some embodiments, the composition comprises an MMI and an anti-tumor preparation. In some embodiments, such a composition further comprises a TKI or a carrier (e.g., as described herein). In some embodiments, the composition comprises an MMI, an anti-tumor preparation, a TKI, and a carrier (e.g., as described herein).

In certain embodiments, the composition comprises an ARB and an antigen. In
certain embodiments, the composition comprises an ARB and a vaccine. In certain embodiments, the composition comprises an ARB and an anti-tumor preparation. In certain embodiments, the composition comprises an ARB, an anti-tumor preparation, and a TKI. In certain embodiments, such compositions further comprises a carrier (e.g., as described herein).

In certain embodiments, the composition comprises a compound of Formula (I) and an antigen. In certain embodiments, the composition comprises a compound of Formula (I) and a vaccine. In certain embodiments, the composition comprises a compound of Formula (I) and an anti-tumor preparation. In certain embodiments, the composition comprises a compound of Formula (I), an anti-tumor preparation, and a TKI. In certain embodiments, such compositions further comprise a carrier (e.g., as described herein).

In certain embodiments, the composition comprises at least two different MMIs and an antigen. In certain embodiments, the composition comprises at least two different MMIs and a vaccine. In certain embodiments, the composition comprises at least two different MMIs and an anti-tumor preparation. In certain embodiments, the composition comprises at least two different MMIs, an antigen, and a TKI. In certain embodiments, the composition comprises at least two different MMIs, a vaccine, and a TKI. In certain embodiments, the composition comprises at least two different MMIs, an anti-tumor preparation, and a TKI. In certain embodiments, such compositions further comprise a carrier (e.g., as described herein).

In certain embodiments the composition comprises two or more monocyte migration inhibitors. Any two or more monocyte migration inhibitors may be combined in a composition of the present invention, and such compositions may also optionally further comprise (i) an antigen, vaccine, or anti-tumor preparation, and/or (ii) a TKI e.g., sunitinib, and/or (iii) a non-specific immunostimulant. In certain embodiments, at least one of the two or more MMIs is described herein. In some instances, it is preferable to combine two or more MMIs having different mechanisms of action for inhibiting monocyte migration into a single composition, as such a combination may in some cases results in a synergistic (i) inhibition of monocyte migration, (ii) enhancement of an immune response, (iii) decrease in monocyte migration to a lymph node, (iv) amplification of vaccine immunity, and (v)
reduction of tumor growth or metastasis in an individual when administered to an individual. Accordingly, the present invention provides for such compositions, e.g., two or more inhibitors from the different classes listed. In certain embodiments the composition comprises two or more monocyte migration inhibitors (MMI) selected from the Class I, the Class II, and the Class III MMIs disclosed herein. In some instances the Class I MMIs are selected from the group consisting of losartan, irbesartan, and telmisartan; the Class II MMI is a compound of Formula (I), (e.g., Ondansetron); and the Class III MMI is a competitive antagonists of the CCR2 receptor (e.g., RS102895, and RS504393). In some embodiments, compositions such as these optionally further comprise a carrier (e.g., as described herein).

In certain embodiments, non-limiting examples of the MMI combinations that the present invention contemplates for inclusion in a single composition include each of the following embodiments described in Table 5:

<table>
<thead>
<tr>
<th>Table 5: Non-limiting examples of MMI combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
</tr>
<tr>
<td>b</td>
</tr>
<tr>
<td>c</td>
</tr>
<tr>
<td>d</td>
</tr>
<tr>
<td>e</td>
</tr>
<tr>
<td>f</td>
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<tr>
<td></td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>g</td>
</tr>
<tr>
<td>h</td>
</tr>
</tbody>
</table>

In some embodiments, any MMI (e.g., the MMIs described herein), or any MMI combination described herein, (e.g., any one of the non-limiting examples of the MMI combinations presented in (a) - (h) in Table 5 herein) is combined into a single composition with one or more general non-specific immunostimulants (e.g., a cationic lipid DNA complex or "CLDC"). In some embodiments, one or more MMIs and one or more general immunostimulants are comprised in a single composition. In some embodiments, the one or more MMIs and the one or more general immunostimulants are comprised in separate compositions. All such compositions are contemplated for use in the methods of the present disclosure, e.g., for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

In still further embodiments any MMI (e.g., the MMIs described herein), or any MMI combination described herein, (e.g., any one of the non-limiting examples of the MMI combinations presented in (a) - (h) in Table 5 herein), optionally combined in a single composition with one or more general immunostimulants (e.g., CLDC), may also be combined in a single composition with one or more receptor tyrosine kinase inhibitor (TKI), e.g., sunitinib or GW2580.

Particular embodiments of the present invention contemplate the combination of sunitinib with any of the MMIs or MMI combinations of the present invention (e.g., any one of the non-limiting examples of the MMI combinations presented in (a) - (h) in Table 5 herein). Still further embodiments contemplate such combinations in a single composition further comprising CLDC. In some embodiments, the one or more MMIs and the one or more TKIs (e.g., sunitinib or GW2580) are comprised in separate compositions. All such
compositions are contemplated for use in the methods of the present disclosure, e.g., for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

In particular embodiments, the present invention contemplates providing the combinations of the present invention (e.g., any one of the non-limiting examples of the MMI combinations presented in (a) - (h) in Table 5 herein) optionally further comprising one or more TKI as supplied in a single dosage form such as is described herein (e.g., a capsule, tablet, powder, or in a liquid dosage form, such as an elixir, syrup, and suspension).

In some embodiments, such a single dosage form is suitable for oral delivery. In some embodiments, the single dosage form for oral delivery that comprises any one of the combinations of the present invention (e.g., any one of the non-limiting examples of the MMI combinations presented in (a) - (h) in Table 5 herein) and optionally further comprises one or more TKI is used to treat a cancer patient, e.g., to reduce or inhibit tumor growth or metastasis.


For oral administration, the compositions of the invention can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed
tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (e.g., at least National Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions intended for in vivo use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under GMP conditions.

**Kits**

The invention also provides kits and articles of manufacture comprising the compounds or compositions described herein for use in the methods of enhancing an immune response, decreasing recruitment of monocytes to a lymph node, amplifying vaccine immunity, or inhibiting tumor growth or metastasis. In particular embodiments, a kit comprises an MMI and an antigen, a vaccine, or an anti-tumor preparation, as disclosed herein. In particular embodiments, a kit comprises an MMI and a TKI (e.g., sunitinib or GW2580). In particular embodiments, a kit comprises an MMI and a non-specific immunostimulant (e.g., CLDC). In particular embodiments, a kit comprises an ARB, an antigen, and optionally a TKI. In particular embodiments, a kit comprises an ARB, a vaccine, and optionally a TKI. In particular embodiments, a kit comprises an ARB, an anti-tumor preparation, and optionally a TKI. In particular embodiments, a kit comprises a
compound of Formula (I), an antigen, and optionally a TKI. In particular embodiments, a kit comprises a compound of Formula (I), a vaccine, and optionally a TKI. In particular embodiments, a kit comprises a compound of Formula (I), an anti-tumor preparation, and optionally a TKI.

In particular embodiments, a kit comprises two or more MMIs and an optionally an antigen, a vaccine, or an anti-tumor preparation. In further embodiments, such a kit may optionally further comprise a TKI (e.g., sunitinib or GW2580) and/or a non-specific immunostimulant e.g., CLDC. In various embodiments, any two or more compounds in a kit may be present in one or more compositions, each composition present in a different container or compartment. In certain embodiments, each compound in a kit is present in a separate composition.

In certain embodiments, kits of the invention include a container or compartment comprising a compound of Formula (I)

![Chemical Structure](image)

(I)

wherein

R\(^1\) is hydrogen or Ci\(_{1-6}\)alkyl;

\(\equiv\equiv\) is a single bond or double bond;

Q\(^1\) is N or CH;

R\(^2\) is selected from hydrogen and Ci\(_{1-6}\)alkylene, wherein one carbon unit of said alkylene is optionally replaced with -O-, -S-, -SO-, -SO\(_2\)-, -NR\(^a\)-, or -CO-; wherein R\(^a\) is hydrogen or Ci\(_{1-6}\)alkyl; and

R\(^3\) is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Ci\(_{1-6}\)alkyl; or pharmaceutically acceptable salts thereof, and an antigen, a vaccine, or anti-tumor preparation. In certain embodiments, kits of the invention include a container or compartment comprising a Compound of Formula (II), Formula (III), Formula (IV) or Formula (V) and an antigen, a vaccine, or an anti-tumor preparation.
preparation. In certain embodiments, the kit includes Ondansetron. In certain embodiments, the kit includes Alosetron. In certain embodiments, kits of the invention provide the compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in a first container or compartment and the antigen, vaccine, or anti-tumor preparation in a second container or compartment. In certain embodiments the kits further comprise other adjuvants. In certain embodiments, the kits of the invention further comprise instructions for use in accordance with any of the methods described herein.

In certain embodiments, kits of the invention include a container or compartment comprising a ARB and an antigen, a vaccine, or anti-tumor preparation. In certain embodiments, kits of the invention include a container or compartment comprising Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsaran, and/or Valsartan and an antigen, a vaccine, or anti-tumor preparation. In certain embodiments, kits of the invention provide an ARB, e.g., Losartan in a first container or compartment and the antigen, vaccine, or anti-tumor preparation in a second container or compartment. In certain embodiments the kits further comprise other adjuvants. In certain embodiments, the kits of the invention further comprise instructions for use in accordance with any of the methods described herein.

In particular embodiments, the combination of (i) ARB or Compound of Formula (I) and (ii) antigen, vaccine or anti-tumor preparation, present in a kit or composition of the present invention (including compositions present within a kit of the present invention) is selected from any of the combinations listed in Table 6 herein. In one certain embodiment, the kit of the invention comprises at least two separate containers, with each separate container comprising at least one of two separate compositions (e.g., pharmaceutically acceptable compositions or formulations), wherein a first of the two compositions comprises an ARB or a Compound of Formula (I) and the second of the two separate compositions comprises an antigen, vaccine, or anti-tumor preparation. In particular embodiments, the combination of the first and the second compositions is selected from any one of the combinations listed in Table 6.
<table>
<thead>
<tr>
<th>Column A: Combination Number</th>
<th>Column B: ARB or Compound of Formula (I)</th>
<th>Column C: Antigen, Vaccine, or Anti-tumor preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ondansetron</td>
<td>Antigen</td>
</tr>
<tr>
<td>2</td>
<td>Ondansetron</td>
<td>Vaccine</td>
</tr>
<tr>
<td>3</td>
<td>Ondansetron</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>4</td>
<td>Ondansetron</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>5</td>
<td>Alosetron</td>
<td>Antigen</td>
</tr>
<tr>
<td>6</td>
<td>Alosetron</td>
<td>Vaccine</td>
</tr>
<tr>
<td>7</td>
<td>Alosetron</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>8</td>
<td>Alosetron</td>
<td>Antigen</td>
</tr>
<tr>
<td>9</td>
<td>Azilsartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>10</td>
<td>Azilsartan</td>
<td>Vaccine</td>
</tr>
<tr>
<td>11</td>
<td>Azilsartan</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>12</td>
<td>Azilsartan</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>13</td>
<td>Candesartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>14</td>
<td>Candesartan</td>
<td>Vaccine</td>
</tr>
<tr>
<td>15</td>
<td>Candesartan</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>16</td>
<td>Candesartan</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>17</td>
<td>Eprosartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>18</td>
<td>Eprosartan</td>
<td>Vaccine</td>
</tr>
<tr>
<td>19</td>
<td>Eprosartan</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>20</td>
<td>Eprosartan</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>21</td>
<td>Irbesartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>22</td>
<td>Irbesartan</td>
<td>Vaccine</td>
</tr>
<tr>
<td>23</td>
<td>Irbesartan</td>
<td>Anti-tumor preparation</td>
</tr>
<tr>
<td>24</td>
<td>Irbesartan</td>
<td>Sunitinib</td>
</tr>
<tr>
<td>25</td>
<td>Losartan</td>
<td>Antigen</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td></td>
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<tr>
<td>---</td>
<td>-------------------</td>
<td>---</td>
</tr>
<tr>
<td>26</td>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Anti-tumor</td>
<td>preparation</td>
</tr>
<tr>
<td>28</td>
<td>Sunitinib</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>Olmesartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>30</td>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Anti-tumor</td>
<td>preparation</td>
</tr>
<tr>
<td>32</td>
<td>Sunitinib</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Telmisartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>34</td>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Anti-tumor</td>
<td>preparation</td>
</tr>
<tr>
<td>36</td>
<td>Sunitinib</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Valsartan</td>
<td>Antigen</td>
</tr>
<tr>
<td>38</td>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Anti-tumor</td>
<td>preparation</td>
</tr>
<tr>
<td>40</td>
<td>Sunitinib</td>
<td></td>
</tr>
</tbody>
</table>

In certain embodiments, the kits of the invention further comprise instructions for use in accordance with any of the methods described herein.

In particular embodiments kits of the invention comprise at least two MMIs. In certain embodiments, the two MMIs are from different classes. In certain embodiments the two MMIs are provided in separate containers or compartments. In certain embodiments, the kits of the present invention provide at least one composition comprising two MMIs selected from the MMI combinations presented in (a) - (h) of Table 5 herein. In some embodiments, such a composition is provided in the kit in a container or a compartment. In some embodiments, such kits optionally further comprise an antigen, vaccine, or anti-tumor preparation provided in a separate container or compartment, or optionally in with the MMIs. In some embodiments, such kits also optionally further comprise an adjuvant. In some embodiments, such kits optionally further comprise a TKI (e.g., sunitinib or GW2580).

In certain embodiments, the kits of the present invention provide at least two MMIs, wherein the combination of the two MMI is any one of the combinations presented in
paragraphs (i)-(p) in the "Method Of Using Two Or More Monocyte Migration Inhibitors" section herein, and wherein the MMIs are provided in at least two separate containers or compartments. In some embodiments, such kits optionally further comprise an antigen, vaccine, or anti-tumor preparation provided in a separate container or compartment, or optionally in with at least one of the MMIs. In some embodiments, such kits also optionally further comprise an adjuvant. In some embodiments, such kits optionally further comprise a TKI (e.g., sunitinib).

Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable. The instructions relating to the use of the compositions or kits described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. In some embodiments, the instructions comprise instructions for administering an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in conjunction with an antigen, a vaccine, or an anti-tumor preparation. In some embodiments, the instructions relate to administering one or more, or two or more, MMIs, or administering one or more MMI in combination with a TKI. Kits of the invention may further comprise a description of selecting an individual suitable or treatment.

The present invention also provides kits comprising compounds or compositions described herein and may further comprise instruction(s) on methods of using the kit or composition, such as uses further described herein. The kit may comprise one or more unit dosages of a composition described herein. The kit may contain instructions for administering an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) and the antigen, vaccine, or anti-tumor preparation simultaneously or sequentially, as described elsewhere herein. In some embodiments, the kit of the invention comprises the packaging described herein. In other embodiments, the kit of the invention comprises the packaging described herein and a second packaging comprising a buffer. The kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein. In certain embodiments, a container or
compartment is a vial, bottle, jar, flexible package (e.g., sealed Mylar or plastic bag), and the like.

Kits may also include multiple unit doses of an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) and an antigen, a vaccine, or an anti-tumor preparation and instructions for use and packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies. The kits of the invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information.

METHODS OF ENHANCING AN IMMUNE RESPONSE AGAINST AN ANTIGEN IN AN INDIVIDUAL

A vaccine can be administered to an individual to elicit an immune response that can lessen the severity and/or duration of a disease or infection. Vaccines can include antigens. However, not all antigens are capable of stimulating a sufficiently robust B cell and/or T cell response to produce protective immunity. Certain individuals, for example, children, the elderly, or the immunocompromised, may not be capable of mounting a robust immune response. The compositions described herein can be used to enhance an immune response in an individual to whom an antigen has been administered. Such methods/uses include administering a composition containing the antigen to the individual in conjunction with an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron). In certain embodiments, the methods include administering a composition containing the antigen to the individual with Ondansetron. In certain embodiments, the methods include administering a composition containing the antigen to the individual with Alosetron. In certain embodiments, the methods include administering a composition containing the antigen to the individual with an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmilsartan, Azilsaran, and/or Valsartan).

In some embodiments, the present invention provides a method of enhancing an immune response against an antigen in an individual, the method comprising administering to the individual the antigen in conjunction with an effective amount of an angiotensin II
receptor blocker (ARB) or a compound of Formula (I):

![Chemical structure](image)

wherein

- $R^1$ is hydrogen or $C_{1-6}$ alkyl;
- $Q^1$ is N or CH;
- $R^2$ is selected from hydrogen and $C_{1-6}$ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -O-, -S-, -SO-, -SO$_2$-, -NR$^a$-, or -CO-; wherein $R^3$ is hydrogen or $C_{1-6}$ alkyl; and
- $R^3$ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with $C_{1-6}$ alkyl;

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (II), Formula (III), or Formula (IV), as described herein. In certain embodiments, the compound of Formula (I) is Ondansteron or Alosetron.

In certain embodiments, the ARB is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan.

In some embodiments, the method further comprises administering a receptor tyrosine kinase inhibitor to the individual in combination with the antigen the ARB or the compound of Formula (I). In one embodiment, the TKI inhibitor is sunitinib or GW2580.

In certain embodiments, the enhanced immune response comprises an enhanced humoral immune response. In certain embodiments, the enhanced humoral immune response comprises an increased antibody titer against the antigen. In certain embodiments, the enhanced immune response comprises an enhanced cellular immune response. In certain embodiments, the enhanced cellular immune response comprises increased release of IFNy in response to the antigen. In certain embodiments, the enhanced immune response...
comprises an enhanced humoral immune response and an enhanced cellular immune response.

In certain embodiments, the antigen comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

In certain embodiments comprising administering an ARB, the individual receiving an ARB does not have hypertension, congestive heart failure, a history of myocardial infarction, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB has not taken the ARB for the treatment of hypertension, congestive heart failure, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB does not have a detectable level of the ARB in their blood or urine prior to administration of the ARB in conjunction with the antigen.

In certain embodiments, the antigen and the ARB or the compound of Formula I are present in a single pharmaceutical composition. In certain embodiments, the single pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intrapleurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

In certain embodiments, the ARB or the compound of Formula I is present in a first pharmaceutical composition and the antigen is present in a second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intrapleurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the second pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally,
transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the first pharmaceutical composition is administered before the second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered after the second pharmaceutical composition. In certain embodiments, the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another. In certain embodiments, the first pharmaceutical composition and the second pharmaceutical compositions are administered simultaneously.

In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of 30 mg/kg. In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of less than 25 mg. In certain embodiments, the ARB is Candesartan, and the Candesartan is administered at a dosage of less than 4 mg. In certain embodiments, the ARB is Eprosartan, and the Eprosartan is administered at a dosage of less than 400 mg. In certain embodiments, the ARB is Irbesartan, and the Irbesartan is administered at a dosage of less than 150 mg. In certain embodiments, the ARB is Olmesartan, and the Olmesartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Telmisartan, and the Telmisartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Valsartan, and the Valsartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Azilsartan, and the Azilsartan is administered at a dosage of less than 80 mg.

In some embodiments, the compound of Formula (I) is a compound of Formula (II):

```
     O
    /   \
   /     \  \n    O1   R4
   /     \  \n  /       \ \
R1       R4
```

(II)

wherein

R1 is hydrogen or C1-6 alkyl;

is a single bond or double bond;

Q1 is N or CH; and

R4 is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl
ring is optionally substituted with \( \text{Ci}_{6} \) alkyl;

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (III):

\[ \text{(III)} \]

wherein

\( \equiv \equiv \) is a single bond or double bond;
\( Q^1 \) is \( \text{N or CH} \); and
\( R^4 \) is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \( \text{Ci}_{6} \) alkyl;

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (IV):

\[ \text{(IV)} \]

wherein

\( R^1 \) is hydrogen or \( \text{Ci}_{6} \) alkyl;
\( Q^1 \) is \( \text{N or CH} \); and

\( R^4 \) is selected from \( \text{and} \) ;

or pharmaceutically acceptable salts thereof.
In some embodiments, the compound of Formula (I) is a compound of Formula (V):

![Chemical Structure]

(V)

wherein

- is a single bond or double bond;

Q<sup>1</sup> is N or CH; and

R<sup>4</sup> is selected from

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is Ondansetron. In some embodiments, the Ondansetron is administered at a dosage of 3 mg/kg. In some embodiments, the Ondansetron is administered at a dosage of less than 12 mg. In some embodiments, the compound of Formula (I) is Alosetron. In some embodiments, the Alosetron is administered at a dosage of less than 0.5 mg.

In some embodiments comprising administering a Ondansetron or Alosetron, the individual has not taken the Ondansetron or the Alosetron for the treatment of irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONY), radiation-induced nausea and vomiting (RINV), or chemotherapy-induced nausea and vomiting (CINV). In some embodiments, the individual does not have a detectable level of the Ondansetron or the Alosetron in their blood or urine prior to administration of the Ondansetron or the Alosetron in conjunction with the antigen.

The compound of Formula (I) administered in conjunction with the antigen can be any compound of Formula (I), combination of compounds of Formula (I), or any prodrug, salt, or derivative of compound of Formula (I) described herein. The ARB administered in conjunction with the antigen can be any ARB, combination of ARBs, or any prodrug, salt, or derivative of an ARB described herein. The antigen administered in conjunction with the ARB or the compound of Formula (I) (e.g., Ondansetron or Alosetron) can be any antigen...
described herein. The ARB or the compound of Formula (I) (e.g., Ondansetron or Alosetron) and the antigen can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing the ARB or the compound of Formula (I) (e.g., Ondansetron or Alosetron) and/or the antigen can be administered according to any method known in the art at dosages described elsewhere herein.

In certain embodiments, the individuals to whom a compound of Formula (I) is administered in conjunction with the antigen are those who are otherwise not receiving the Ondansetron or Alosetron for the treatment of, e.g., irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), chemotherapy-induced nausea and vomiting (CINV), radiation-induced nausea and vomiting (RINV), and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or is otherwise in need of treatment, can receive Ondansetron and/or Alosetron in conjunction with an antigen for the purpose of enhancing an immune response if the individual is not being treated with Ondansetron and/or Alosetron. Alternatively, the individuals to whom a compound of Formula (I) is administered in conjunction with the antigen can be those who have temporarily suspended Ondansetron and/or Alosetron treatment and have been shown, using methods well known in the art, to not have detectable levels of Ondansetron and/or Alosetron in their blood and/or urine prior to the administration of the Ondansetron and/or Alosetron in conjunction with the antigen. Details regarding such techniques are described in, e.g., Somers et al. (2007) Xenobiotica 37: 855-869; Koch et al. (2002) Br J Clin Pharmacol. 53: 238-242; Xu et al. (2000) J Mass Spectrom 35: 1329-1334; Roila et al. (1995) Clin Pharmacokinet 29: 95-103; and others.

In certain embodiments, the individuals to whom an ARB is administered in conjunction with an antigen can be those who are otherwise not receiving an ARB for treatment of a pre-existing condition, e.g., hypertension, diabetic nephropathy, heart failure, and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or who is otherwise in need of treatment, can receive an ARB in conjunction with an antigen for the purpose of enhancing an immune response if
the individual is not being treated with an ARB. Alternatively, the individuals to whom an ARB is administered in conjunction with an antigen can be those who have temporarily suspended ARB treatment and have been shown, using methods well known in the art, to not have detectable levels of an ARB in their blood and/or urine prior to the administration of the ARB in conjunction with the antigen. Details regarding such techniques are described in, e.g., Nakashima et al. (1996) Blood Press. Suppl. 2: 62-66; Sica et al. (2005) Clin. Pharmacokinet. 44(8): 797-814; Lu et al. (2011) J. Pharm. Biomed. Anal. 54(1): 100-105; Yeung et al. (2000) Int. J. Pharmaceut. 204: 17-22; Chando et al. (1998) Drug Metab. Dispos. 26(5): 408-417; McCarthy et al. (1998) J. Pharm Biomed. Anal. 17: 671-677; Ferreiros et al. (2007) Ther. Drug Monitoring 29(6): 824-834; Gonzalez et al. (2002) J. Chromatography A 949: 49-60; and others.

**Detecting Enhanced Immune Responses**

In certain embodiments, the methods of the invention can be used to enhance a humoral immune response (i.e., B cell response) and/or a cellular response (i.e., T cell response). An enhanced humoral immune response can be demonstrated by showing that the antibody titers against a specific antigen from, e.g., an individual who received an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in conjunction with the antigen, are higher than antibody titers against the antigen from, e.g., an individual who received only the antigen. Antibody levels against a specific antigen can be determined via solid phase radioimmunoassay (RIA), in which serially diluted blood serum is incubated in microtiter wells previously coated with the antigen administered to the individual in conjunction with an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron). Bound antibody is detected by employing 125I-labeled anti-immunoglobulin antibodies. The amount of specific antibody in the antiserum is then determined from a standard curve generated with a specific antibody of known concentration. Another method by which antibody titers can be determined is ELISA, in which an enzyme-conjugated secondary antibody, rather than radiolabeled secondary antibody, is used to detect the binding of primary antibodies to the antigen. Such methods are well known to those of skill in the art and are described in further detail in, e.g., Essentials of Immunology & Serology (Stanley, 2002); Clinical Immunology & Serology: A Laboratory Perspective (Stevens,

An enhanced cellular immune response can be demonstrated by showing that T cells from, e.g., an individual who received an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in conjunction with a specific antigen, are more highly activated by the antigen than T cells from, e.g., an individual who received only the antigen. One of the most common ways to assess T cell activation is to measure T-cell proliferation or T-cell cytokine elaboration upon in vitro stimulation of T-cells by the antigen administered in the methods (e.g., whole antigen or fragments thereof). This can be assessed via, e.g., ELISpot, a sensitive colorimetric assay based on ELISA that can detect secreted cytokine at the single cell level, rather than antigen-bound antibody. Details regarding ELISpot and related techniques (such as FLUROspot) are described in, e.g., Tobery et al. (2001) J. Immunol. Meth. 254: 59-66; Braun et al. (2006) Virology J. 3:53-68; Davis, et al. (2004) PNAS 101(29) 10697-10702; Posavad, et al. (201) Vaccine 29(40): 7058-7066; Hutchings et al. (1989) J Immunol Methods. 120: 1-8; and others. ELISpot kits are also commercially available from, e.g., MABTECH AB, R&D Systems, BD Biosciences, ABCam, and other manufacturers.

T cell activation can also be assayed via flow cytometry. For example, T-cells can stimulated with, e.g., a protein, peptide, or group of peptides derived from the antigen administered during the methods, and cultured for a period of time. During that time, an inhibitor is added which blocks the release of cytokines from the T cells. The T cells are then fixed and permeabilized to allow anti-cytokine-specific antibodies to stain the intracellular cytokines, allowing them to be visualized during FACS analysis in relatively high quantity. Further details regarding this technique are described in, e.g., Caruso et al. (1997) Cytometry 27(1): 71-76; Nomura et al. (2000) Cytometry. 40: 60-68; Suni et al. (1998) J Immunol Methods. 212: 89-98; and others.
An individual to whom an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) is administered in conjunction with an antigen can exhibit a humoral immune response that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90-fold, at least about 100-fold, at least about 110-fold, at least about 125-fold, at least about 150-fold, at least about 175-fold, at least about 200-fold, or more than 200-fold (e.g., about 250-fold, about 300-fold, or about 350-fold) higher than the humoral immune response exhibited by an individual to whom vaccine alone is administered, including any range in between about 2-fold and about 300-fold. An individual to whom an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) is administered in conjunction with an antigen can exhibit a cellular immune response that is at least about 1.2-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, or more than about 5-fold (e.g., about 5.5-fold, about 6-fold, or about 6.5-fold) higher than the cellular immune response exhibited by an individual to whom vaccine alone is administered, including any range between about 1.2-fold and about 6.5-fold.

**Antigens**

The antigen is administered in conjunction with an MMI (such as an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)) or a combination of such MMIs in any suitable amount that is sufficient to generate an enhanced immune response. The antigen administered in conjunction with (such as an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)) or a combination of such MMIs can be any antigen or combination of antigens described herein. The amount of antigen to be included in the compositions and used in the methods of the present invention (i.e., any of the methods described herein) will depend on the immunogenicity of the antigen itself and the efficacy of any adjuvants co-administered therewith. In general, an immunologically effective dose comprises between about 1 µg to about 1000 µg of the antigen, preferably between about 5 µg to about 500 µg, more preferably between about 10 µg to about 200 µg. In some embodiments, an immunologically
effective dose can be at least about 1 µg, at least about 5 µg, at least about 10 µg, at least about 25 µg, at least about 50 µg, at least about 100 µg, at least about 150 µg, at least about 200 µg, at least about 250 µg, at least about 300 µg, at least about 350 µg, at least about 400 µg, at least about 450 µg, at least about 500 µg, at least about 550 µg, at least about 600 µg, at least about 650 µg, at least about 700 µg, at least about 750 µg, at least about 800 µg, at least about 850 µg, at least about 950 µg, or up to about 1000 µg of antigen. In embodiments where the antigen is a recombinant protein or peptide, a suitable dose can be about 10-100 µg. In embodiments where the antigen is a recombinant protein or peptide, a suitable dose can be about 10-100 µg.

METHODS OF DECREASING MONOCYTES RECRUITMENT TO A LYMPH NODE

Vaccination can trigger the mobilization and recruitment of antigen presenting cells (APC) to lymph nodes, e.g., vaccine draining lymph nodes. Monocytes, one subtype of APC, have recently been shown to have a suppressive effect on B cell and T cell proliferation, thus limiting immune responses during vaccination. The present invention is based in part on the observation that the administration of an effective amount of an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in conjunction with an antigen decreases the recruitment of monocytes, e.g., CD14<sup>+</sup>CD16<sup>+</sup> human monocytes, to a lymph node.

In some embodiments, the present invention provides a method of decreasing recruitment of monocytes to a lymph node in an individual, the method comprising administering to the individual an antigen in conjunction with an effective amount of an ARB or a compound of Formula (I):

![Formula (I)](attachment:image)

wherein

R<sup>1</sup> is hydrogen or C<sub>1-6</sub> alkyl;

== is a single bond or double bond;
Q\(^1\) is N or CH;
R\(^2\) is selected from hydrogen and \(\text{Ci}_6\) alkylene, wherein one carbon unit of said alkylene is optionally replaced with -O-, -S-, -SO-, -SO\(_2\)-, -NR\(^a\)-, or -CO-; wherein R\(^3\) is hydrogen or \(\text{Ci}_6\) alkyl; and

R\(^3\) is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \(\text{Ci}_6\) alkyl; or pharmaceutically acceptable salts thereof. In some embodiments, the compound of Formula (I) is a compound of Formula (II), Formula (III), or Formula (IV), as described herein. In certain embodiments, the compound of Formula (I) is Ondansteron or Alosetron.

In certain embodiments, the ARB is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan.

In certain embodiments, the monocytes are inflammatory monocytes or CD14\(^hi\)CD16\(^+\) human monocytes. In certain embodiments, the lymph node is a draining lymph node. In certain embodiments, the draining lymph node is a vaccine draining lymph node.

In certain embodiments, the enhanced immune response comprises an enhanced humoral immune response. In certain embodiments, the enhanced humoral immune response comprises an increased antibody titer against the antigen. In certain embodiments, the enhanced immune response comprises an enhanced cellular immune response. In certain embodiments, the enhanced cellular immune response comprises increased release of IFNy in response to the antigen. In certain embodiments, the enhanced immune response comprises an enhanced humoral immune response and an enhanced cellular immune response.

In certain embodiments, the antigen comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

In certain embodiments comprising administering an ARB, the individual receiving an ARB does not have hypertension, congestive heart failure, a history of myocardial infarction, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB has not taken the ARB for the treatment of hypertension, congestive heart failure, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB does not have a detectable level of the ARB in their blood or urine prior to administration of the ARB.
in conjunction with the antigen.

In certain embodiments, the antigen and the ARB or the compound of Formula I are present in a single pharmaceutical composition. In certain embodiments, the single pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

In certain embodiments, the ARB or the compound of Formula I is present in a first pharmaceutical composition, and the antigen is present in a second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the second pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the first pharmaceutical composition is administered before the second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered after the second pharmaceutical composition. In certain embodiments, the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another. In certain embodiments, the first pharmaceutical composition and the second pharmaceutical compositions are administered simultaneously.

In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of 30 mg/kg. In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of less than 25 mg. In certain embodiments, the ARB is
Candesartan, and the Candesartan is administered at a dosage of less than 4 mg. In certain embodiments, the ARB is Eprosartan, and the Eprosartan is administered at a dosage of less than 400 mg. In certain embodiments, the ARB is Irbesartan, and the Irbesartan is administered at a dosage of less than 150 mg. In certain embodiments, the ARB is Olmesartan, and the Olmesartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Telmisartan, and the Telmisartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Valsartan, and the Valsartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Azilsartan, and the Azilsartan is administered at a dosage of less than 80 mg.

In some embodiments, the compound of Formula (I) is a compound of Formula (II):

\[
\text{(II)}
\]

wherein

- \( R^1 \) is hydrogen or \( \text{C}_{1-6} \) alkyl;
- \( \equiv \) is a single bond or double bond;
- \( Q^1 \) is \( \text{N} \) or \( \text{CH} \);
- \( R^4 \) is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \( \text{C}_{1-6} \) alkyl;
- or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (III):

\[
\text{(III)}
\]

wherein

- \( \equiv \) is a single bond or double bond;
Q^1 is N or CH; and

R^4 is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C_{1-6} alkyl; or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (IV):

![Chemical Structure](attachment:image.png)

wherein
R^1 is hydrogen or C_{1-6} alkyl;

Q^1 is N or CH; and

R^4 is selected from some structures and or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (V):

![Chemical Structure](attachment:image.png)

wherein

Q^1 is N or CH; and

R^4 is selected from some structures and or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is Ondansetron. In some
embodiments, the Ondansetron is administered at a dosage of 3mg/kg. In some embodiments, the Ondansetron is administered at a dosage of less than 12 mg. In some embodiments, the compound of Formula (I) is Alosetron. In some embodiments, the Alosetron is administered at a dosage of less than 0.5 mg.

In some embodiments, comprising administering a Ondansetron or Alosetron, the individual has not taken the Ondansetron or the Alosetron for the treatment of irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONY), radiation-induced nausea and vomiting (RINV), or chemotherapy-induced nausea and vomiting (CINV). In some embodiments, the individual does not have a detectable level of the Ondansetron or the Alosetron in their blood or urine prior to administration of the Ondansetron or the Alosetron in conjunction with the antigen.

In particular embodiments, methods of decreasing recruitment of monocytes to a lymph node provided by the invention include administering an effective amount of one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, to an individual in conjunction with an antigen. In certain embodiments, an individual to whom one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, is administered in conjunction with an antigen can exhibit a decrease in the number of monocytes that have migrated to lymph node. In certain embodiments, the decrease in the number of monocytes can be at least about 5% less, at least about 10% less, at least about 15% less, at least about 20% less, at least about 25% less, at least about 30% less, at least about 35% less, at least about 40% less, at least about 45% less, at least about 50% less, or more than about 50% less (e.g., about 55%, about 60% or about 65% less) relative to the number of monocytes that have migrated to a lymph node of an individual that has received the antigen alone, including any range between about 5% less and about 65% less. In certain embodiments, the number of monocytes that have migrated to a lymph node in an individual to whom an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) is administered in conjunction with an antigen can be more than 10% less, more than 20% less, more than 30% less, more than 40% less, more than 50% less, more than 60% less, more than about 65% less, more than about 70% less, or more than
about 75% less, more than 80% less, more than about 81% more than about 82% less, more than 83% less, more than 84% less, more than 85% less, more than 86% less, more than 87% less, more than 88% less, more than 89% less, more than 90% less, at least about 91% less, at least about 93% less, or at least about 95% less than the number of monocytes that have migrated to a lymph node of an individual that has received the antigen alone, including any range between about 10% less and about 95% less.

In some embodiments, the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof is administered in conjunction with the antigen can be any compound, combination of compounds, or any prodrug, salt, or derivative of a compound described herein. In some embodiments, Ondansetron can be administered in conjunction with the antigen. In some embodiments, Alosetron can be administered in conjunction with the antigen. In some embodiments, Losartan can be administered in conjunction with the antigen. In certain embodiments, the antigen administered in conjunction with the MMI (such as an ARB, a CCR2 antagonist, or a compound of Formula (I)) can be any antigen described herein, and can be provided in any amount described herein. The MMI (such as an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)) and the antigen can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, and/or the antigen can be administered according to any method known in the art at dosages described elsewhere herein.

In certain embodiments, the individuals to whom a compound of Formula (I) is administered in conjunction with the antigen can be those who are otherwise not receiving Ondansetron or Alosetron for the treatment of, e.g., irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), chemotherapy-induced nausea and vomiting (CINV), radiation-induced nausea and vomiting (RINV), and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease
described herein, or is otherwise in need of treatment, can receive Ondansetron and/or Alosetron in conjunction with an antigen for the purpose of enhancing an immune response if the individual is not being treated with Ondansetron and/or Alosetron. Alternatively, in certain embodiments, the individuals who are being treated with Ondansetron and/or Alosetron can temporarily suspend Ondansetron and/or Alosetron treatment. For example, an individual who is being treated with Ondansetron and/or Alosetron for a pre-existing condition can receive Ondansetron and/or Alosetron in conjunction with an antigen if the individual has taken Ondansetron and/or Alosetron for treatment of the pre-existing condition more than about 10 minutes, more than about 30 minutes, more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than about 24 hours, more than about 1 day more than about 2 days, more than about 3 days, more than about 4 days, more than about 5 days, more than about 6 days, more than about 1 week, more than about 3 weeks, more than about 1 month, more than about 2 months, more than about three months, more than about 4 months, more than about 5 months, more than about 6 months, more than about 7 months, more than about 8 months, more than about 9 months, more than about 10 months, more than about 11 months, or more than about 1 year before receiving the Ondansetron and/or Alosetron in conjunction with the antigen, including any range in between these values. Alternatively, the individuals to whom a compound of Formula (1) is administered in conjunction with an antigen can be those who have temporarily suspended Ondansetron and/or Alosetron treatment and have been shown, using methods well known in the art, to not have detectable levels of Ondansetron and/or Alosetron in their blood and/or urine prior to the administration of Ondansetron and/or Alosetron in conjunction with the antigen.

In certain embodiments, the individuals to whom an ARB is administered in conjunction with an antigen can be those who are otherwise not receiving an ARB for treatment of a pre-existing condition, e.g., hypertension, diabetic nephropathy, heart failure, and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or who is otherwise in need of treatment, can receive an ARB in conjunction with an antigen for the purpose of enhancing an immune response if the individual is not being treated with an ARB. Alternatively, in certain embodiments, the
individuals to whom an ARB is administered in conjunction with an antigen can be those who have temporarily suspended ARB treatment. For example, an individual who is being treated with an ARB for a pre-existing condition can receive an ARB in conjunction with an antigen if the individual has taken the ARB for treatment more than about 10 minutes, more than about 30 minutes, more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than about 24 hours, more than about 1 day more than about 2 days, more than about 3 days, more than about 4 days, more than about 5 days, more than about 6 days, more than about 1 week, more than about 3 weeks, more than about 1 month, more than about 2 months, more than about three months, more than about 4 months, more than about 5 months, more than about 6 months, more than about 7 months, more than about 8 months, more than about 9 months, more than about 10 months, more than about 11 months, or more than about 1 year before receiving the ARB in conjunction with the antigen, including any range in between these values. Alternatively, an individual who is being treated with an ARB for a pre-existing condition can suspend ARB treatment and be tested, using methods well known in the art, to determine that the individual does not have a detectable level of an ARB in their blood and/or urine prior to the administration of the ARB in conjunction with the antigen.

**Monocytes**

Monocytes are agranular leukocytes that originate in the bone marrow and are released to the peripheral circulation as non-dividing cells. Monocytes constitute approximately 10% of peripheral leukocytes in humans and approximately 4% of leukocytes in mice. Monocytes are equipped with chemokine receptors and adhesion receptors that mediate migration from blood to tissues during infection, where they engulf pathogens and produce immune effector molecules. They can also differentiate into inflammatory dendritic cells or macrophages during inflammation.

In mice, circulating monocytes can be classified into two distinct populations, inflammatory monocytes and resident monocytes, based on the expression of specific cell surface markers. Murine inflammatory monocytes are categorized as CCR2⁺, CX3CR1⁻, and GR1⁺ (also known as Ly6). The monocyte cell population found to suppress vaccine immunity primarily includes CCR2⁺ monocytes Mitchell et al. (2012) *J. Immunology* 189:
5612-5621. Human monocyte subpopulations have also been identified based on the
differential expression of the antigenic markers CD16 and CD14. The human monocytes
that correspond to murine inflammatory monocytes are categorized as CD14\(^{\text{high}}\)CD16\(^{-}\).

**Detecting A Reduction Of Monocyte Recruitment To A Lymph Node**

Vaccines can induce inflammation, which typically results in the recruitment of
monocytes to the site of vaccination and to the lymph nodes, i.e., lymph nodes that lie
immediately downstream of the vaccination site, or "vaccine draining lymph nodes."

Decreased monocyte migration to a lymph node can be demonstrated by showing that the
number of monocytes in, e.g., a vaccine draining lymph node of a human individual who
received one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of
Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, in conjunction
with the antigen, is lower than the number of monocytes in, e.g., a vaccine draining lymph
node of a human individual who received only the antigen. This can be assayed by
performing flow cytometry on tissue obtained from each individual's lymph nodes and, e.g.,
determining whether fewer CD14\(^{\text{hi}}\)CD16\(^{-}\) monocytes are detected in the tissue from the
individual who received the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or
a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof,
in conjunction with the antigen than in the tissue from an individual who did not receive the
one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I)
(e.g., Ondansetron and/or Alosetron), or a combination thereof. In certain embodiments,
decreased monocyte migration to a lymph node, e.g., a vaccine draining lymph node, can be
demonstrated by detecting a change in the circulating levels of, e.g., total monocytes or
inflammatory monocyte, within the first 24 hours after the one or more MMIs such as, e.g.,
an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or
Alosetron), or a combination thereof, is administered to the individual in conjunction with
the antigen.
Methods of determining the amount of circulating CD14<sup>+</sup>CD16<sup>−</sup> monocytes or circulating inflammatory monocytes are known in the art. For example, blood specimens can be collected from individuals and prepared for flow cytometry using labeled antibodies against CD14<sup>+</sup>CD16<sup>−</sup> monocyte-specific cell surface markers or against inflammatory monocyte cell surface markers, such as those described elsewhere herein. Absolute numbers of monocytes can be calculated using leukocyte counts derived from an automated blood cell counter. Additional methods of determining levels of circulating monocytes that can be used are described in, e.g., Aldonyte et al. (2003) Resp. Res. 4:1 1; Janciauskiene et al. (2001) Atherosclerosis 158: 41-51; Nockher et al. (1998) Infect Immun. 66: 2782-2790; and others.

METHODS OF AMPLIFYING VACCINE IMMUNITY

Vaccines have played a key role in reducing the incidence of debilitating and/or fatal diseases. Vaccine immunity depends on the ability of individuals to mount a robust immune response. As noted above, inflammatory monocytes are rapidly recruited to the site of vaccination and to lymph nodes, where they suppress B cell and T cell responses. This a particular public health concern in, e.g., vulnerable populations that exhibit reduced immune responsiveness, such as the young, the elderly, and the immunocompromised. The invention provides methods that can be beneficially used to substantially amplify vaccine immunity. As used herein, "amplifying vaccine immunity" refers to increasing a vaccine-induced immune response, e.g., a vaccine-induced humoral (or B-cell) immune response and/or a vaccine-induced cellular (or T-cell) immune response.

In some embodiments, the present invention provides a method of amplifying vaccine immunity in an individual, the method comprising administering to the individual a vaccine in conjunction with an effective amount of an ARB or a compound of Formula (I):

![Chemical Structure](image)

wherein

R<sup>1</sup> is hydrogen or C<sub>1-6</sub> alkyl;
\[
\text{\textemdash} \text{is a single bond or double bond;}
\]
\[Q^1 \text{is N or CH;}
\]
\[R^2 \text{is selected from hydrogen and Ci}_{6} \text{alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO}_2-, -NR^2-, \text{or -CO-; wherein R}^3 \text{is hydrogen or Ci}_{6} \text{alkyl; and}
\]
\[R^3 \text{is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Ci}_{6} \text{alkyl;}
\]
or pharmaceutically acceptable salts thereof. In some embodiments, the compound of Formula (I) is a compound of Formula (II), Formula (III), or Formula (IV). In certain embodiments, the compound of Formula (I) is Ondansteron or Alosetron.

In certain embodiments, the ARB is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan.

In certain embodiments, the vaccine comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

In certain embodiments comprising administering an ARB, the individual receiving an ARB does not have hypertension, congestive heart failure, a history of myocardial infarction, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB has not taken the ARB for the treatment of hypertension, congestive heart failure, or diabetic nephropathy. In certain embodiments, the individual receiving an ARB does not have a detectable level of the ARB in their blood or urine prior to administration of the ARB in conjunction with the antigen.

In certain embodiments, the antigen and the ARB or the compound of Formula I are present in a single pharmaceutical composition. In certain embodiments, the single pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra- arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

In certain embodiments, the ARB or the compound of Formula I is present in a first
pharmaceutical composition and the antigen is present in a second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the second pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally. In certain embodiments, the first pharmaceutical composition is administered before the second pharmaceutical composition. In certain embodiments, the first pharmaceutical composition is administered after the second pharmaceutical composition. In certain embodiments, the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another. In certain embodiments, the first pharmaceutical composition and the second pharmaceutical compositions are administered simultaneously.

In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of 30 mg/kg. In certain embodiments, the ARB is Losartan, and the Losartan is administered at a dosage of less than 25 mg. In certain embodiments, the ARB is Candesartan, and the Candesartan is administered at a dosage of less than 4 mg. In certain embodiments, the ARB is Eprosartan, and the Eprosartan is administered at a dosage of less than 400 mg. In certain embodiments, the ARB is Irbesartan, and the Irbesartan is administered at a dosage of less than 150 mg. In certain embodiments, the ARB is Olmesartan, and the Olmesartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Telmisartan, and the Telmisartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Valsartan, and the Valsartan is administered at a dosage of less than 20 mg. In certain embodiments, the ARB is Azilsartan, and the Azilsartan is administered at a dosage of less than 80 mg.
In some embodiments, the compound of Formula (I) is a compound of Formula (II):

\[
\begin{align*}
\text{(II)} \\
\text{wherein} \\
\text{R}^1 \text{ is hydrogen or } \text{Ci}_6 \text{ alkyl;} \\
\text{is a single bond or double bond;} \\
\text{Q}^1 \text{ is } \text{N or CH;} \text{ and} \\
\text{R}^4 \text{ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Ci}_6 \text{ alkyl;} \\
\text{or pharmaceutically acceptable salts thereof.}
\end{align*}
\]

In some embodiments, the compound of Formula (I) is a compound of Formula (III):

\[
\begin{align*}
\text{(III)} \\
\text{wherein} \\
\text{is a single bond or double bond;} \\
\text{Q}^1 \text{ is } \text{N or CH;} \text{ and} \\
\text{R}^4 \text{ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Ci}_6 \text{ alkyl;} \\
\text{or pharmaceutically acceptable salts thereof.}
\end{align*}
\]
In some embodiments, the compound of Formula (I) is a compound of Formula (IV):

![Chemical Structure](image)

wherein

- $R^1$ is hydrogen or C$_{1-6}$ alkyl;
- $Q^1$ is N or CH; and

- $R^4$ is selected from ![Chemical Structure](image) and ![Chemical Structure](image);

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is a compound of Formula (V):

![Chemical Structure](image)

wherein

- $\equiv$ is a single bond or double bond;
- $Q^1$ is N or CH; and

- $R^4$ is selected from ![Chemical Structure](image) and ![Chemical Structure](image);

or pharmaceutically acceptable salts thereof.

In some embodiments, the compound of Formula (I) is Ondansetron. In some embodiments, the Ondansetron is administered at a dosage of 3mg/kg. In some embodiments, the Ondansetron is administered at a dosage of less than 12 mg. In some embodiments, the compound of Formula (I) is Alosetron. In some embodiments, the Alosetron is administered at a dosage of less than 0.5 mg.
In some embodiments, comprising administering a Ondansetron or Alosetron, the individual has not taken the Ondansetron or the Alosetron for the treatment of irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), radiation-induced nausea and vomiting (RINV), or chemotherapy-induced nausea and vomiting (CINV). In some embodiments, the individual does not have a detectable level of the Ondansetron or the Alosetron in their blood or urine prior to administration of the Ondansetron or the Alosetron in conjunction with the antigen

In certain embodiments, the methods include administering one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, to an individual in conjunction with a vaccine. An amplified humoral (or B-cell) immune response can be demonstrated by showing that the antibody titers from, e.g., an individual who received one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof in conjunction with the vaccine, are higher than antibody titers from, e.g., an individual who received only the vaccine. An individual to whom one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, was administered in conjunction with an antigen can exhibit a humoral immune response that is at least about 2-fold, at least about 5-fold, at least about 10-fold, at least about 20-fold, at least about 50-fold, at least about 60-fold, at least about 70-fold, at least about 80-fold, at least about 90 fold, at least about 100-fold, at least about 110-fold, at least about 125-fold, at least about 150-fold, at least about 175-fold, at least about 200-fold, or more than 200-fold (e.g., about 250-fold, about 300-fold, or about 350-fold) higher than the humoral immune response exhibited by an individual to whom vaccine alone was administered, including any range in between about 2-fold and about 300-fold. An individual to whom one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or a combination thereof, was administered in conjunction with an antigen can exhibit a cellular immune response that is at least about 1.2-fold, at least about 1.5-fold, at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5 fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, or more than about
5-fold (e.g., about 5.5-fold, about 6-fold, or about 6.5-fold) higher than the cellular immune response exhibited by an individual to whom vaccine alone was administered, including any range between about 1.2-fold and about 6.5 fold.

The compound of Formula (I) administered in conjunction with the vaccine can be any compound, combination of compounds, or any prodrug, salt, or derivative of a compound described herein. In some embodiments, the vaccine can be administered in conjunction with Ondansetron. In some embodiments, the vaccine can be administered in conjunction with Alosetron. The compound of Formula (I) and the vaccine can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing the compound of Formula (I) and/or the vaccine can be administered according to any method known in the art at dosages described elsewhere herein.

The ARB administered in conjunction with the vaccine can be any ARB, combination of ARBs, or any prodrug, salt, or derivative of an ARB described herein. The ARB and vaccine can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing the ARB and/or the vaccine can be administered according to any method known in the art at dosages described elsewhere herein.

The one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) administered in conjunction with the vaccine can be any MMIs such as, e.g., any ARB, CCR2 antagonist, or Compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, or any prodrug, salt, or derivative of an ARB described herein. The one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof and vaccine can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist,
or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof and/or the vaccine can be administered according to any method known in the art at dosages described elsewhere herein.

The individuals to whom an a compound of Formula (I) is administered in conjunction with the vaccine can be those who are otherwise not receiving Ondansetron or Alosetron for the treatment of, e.g., irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), chemotherapy-induced nausea and vomiting (CINV), radiation-induced nausea and vomiting (RINV), and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or is otherwise in need of treatment, can receive Ondansetron and/or Alosetron in conjunction with a vaccine for the purpose of enhancing an immune response if the individual is not being treated with Ondansetron and/or Alosetron. Alternatively, the individuals on whom the methods are practiced have temporarily suspended Ondansetron and/or Alosetron treatment and have been shown, using methods well known in the art, to not have detectable levels of Ondansetron and/or Alosetron in their blood and/or urine prior to the administration of the Ondansetron and/or Alosetron in conjunction with the vaccine.

The individuals to whom an ARB is administered in conjunction with a vaccine can be those who are otherwise not receiving an ARB for treatment of a pre-existing condition, e.g., hypertension, diabetic nephropathy, heart failure, and/or other conditions, as described herein. In another embodiment, an individual who has a condition or disease described herein, or who is otherwise in need of treatment, can receive an ARB in conjunction with a vaccine for the purpose of enhancing an immune response if the individual is not being treated with an ARB. Alternatively, the individuals to whom an ARB is administered in conjunction with a vaccine can be those who have temporarily suspended ARB treatment. For example, an individual who is being treated with an ARB for a pre-existing condition can receive an ARB in conjunction with a vaccine if the individual has taken the ARB for treatment more than about 10 minutes, more than about 30 minutes, more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than about 24 hours, more than about 1 day more than about 2 days, more than about 3 days, more than about 4 days, more than about 5 days, more than about 6
days, more than about 1 week, more than about 3 weeks, more than about 1 month, more
than about 2 months, more than about three months, more than about 4 months, more than
about 5 months, more than about 6 months, more than about 7 months, more than about 8
months, more than about 9 months, more than about 10 months, more than about 11 months,
or more than about 1 year before receiving the ARB in conjunction with the vaccine,
including any range in between these values. Alternatively, an individual who is being
treated with an ARB for a pre-existing condition can suspend ARB treatment and be tested,
using methods well known in the art, to determine that the individual does not have a
detectable level of an ARB in their blood and/or urine prior to the administration of the ARB
in conjunction with the vaccine.

**Vaccines**

A vaccine is a composition that contains an antigen which, when administered to an
individual, stimulates an immune response. Vaccines confer long-term immunity by
inducing the development of immune memory cells that are able to mount a strong response
if the pathogen is detected again. The vaccine that is administered in the methods can be any
vaccine formulation known to those of skill in the art. For example, the vaccine
administered in conjunction with one or more MMIs such as, e.g., an ARB, a CCR2
antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or
combination thereof can contain any antigen known in the art. In certain embodiments, the
vaccine administered in conjunction with one or more MMIs such as, e.g., an ARB, a CCR2
antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or
combination thereof can contain any antigen or combination of antigens described herein,
e.g., a live infectious agent, a killed infectious agent, a polysaccharide, or a toxin produced
by an infectious agent. In certain embodiments of the methods, the vaccine contains specific
proteins, e.g., purified from an infection agent or recombinantly produced. In certain
embodiments of the methods, the polysaccharide or protein present in the vaccine is
conjugated to an immuno-stimulating molecule, such as a carrier protein.

The vaccine administered in conjunction with the one or more MMIs such as, e.g., an
ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or
Alosetron)), or combination thereof can be a DNA vaccine, which entails direct introduction
into appropriate tissues of, e.g., a plasmid encoding the antigen(s) against which an immune response is sought, and which relies on the in situ production of the target antigen. Further details regarding DNA vaccines are described in, e.g., DNA Vaccines: Methods and Protocols (Douglas B. Lourie and Robert Whalen, eds., 2000) Humana Press; DNA Vaccines (Mark Saltzman, Hong Shen, and Janet Brandsma, eds. 2006) Human Press; and others.

The vaccine administered in conjunction with one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof can be a viral vector vaccine, which is typically a live attenuated virus that is genetically engineered to carry DNA encoding protein antigens from an unrelated organism. Viral vector vaccines carry DNA into a host cell for production of antigenic proteins that can be tailored to stimulate an immune response. Viral vector vaccines, unlike DNA vaccines, also have the potential to actively invade host cells and replicate, much like a live attenuated vaccine, further activating the immune system like an adjuvant. Further details regarding viral vector vaccines are described in, I. Brave et al. (2007) Mol Pharm. 4: 18-32; Kaufmann et al. (2012) Trends Mol Med. 18: 365-7; Ulmer et al. (2012) Vaccine. 30: 4414-4418; and others.

In certain embodiments, the vaccine administered in conjunction with one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof can be a dendritic cell vaccine. Dendritic cells (DCs) are antigen-presenting cells that are involved in the induction of primary immune responses. The unique ability of DCs to activate naive and memory CD4+ and CD8+ T cells suggests that they could be used, e.g., for the induction of a specific anti-tumor immunity. An individual's DCs can be harvested, pulsed with one or more antigens, and used to re-immunize the individual to induce a protective immune response. Further details regarding dendritic cell vaccines are described in, e.g., Pellegatta et al. (2009) Methods Mol Biol. 568: 233-247; Bhargava et al. (2012) Immunotherapy. 4: 703-718; Van Brussel et al. (2012) Mediators Inflamm. Article ID 690643; Yamanaka et al. (2012) Adv Exp Med Biol. 746:187-200; and others.
METHODS OF INHIBITING TUMOR GROWTH OR METASTASIS

Myeloid cells including monocytes are known to promote seeding and growth of tumor metastases in the lungs. Therapeutic cancer vaccines stimulate the immune system to recognize tumor-specific antigens and to generate an immune response to find and destroy cells that express them. Producing effective therapeutic vaccines has been difficult, as genetically unstable cancer cells are capable of evading immune recognition. Recent evidence indicates that monocyte depletion can increase the efficacy of cancer vaccine (Mitchell et al. (2012) J. Immunology 189: 5612-5621 and U.S. Patent Application Publication No. US 2012/0156280).

In some embodiments, the present invention provides a method of suppressing tumor growth or metastasis in an individual with cancer, the method comprising administering to the individual one or more monocyte migration inhibitors (MMI). In some embodiments, the MMI is selected from the group consisting of Class I MMIs which have angiotensin receptor blocking activity, Class II MMIs which have serotonin receptor antagonist activity, and Class III MMIs which have CCR2 receptor antagonist activity. In some embodiments the Class I MMIs are selected from the group consisting of losartan, irbesartan, and telmisartan; the Class II MMI is a compound of Formula (I), (e.g., Ondansetron); and the Class III MMI is a competitive antagonists of the CCR2 receptor (e.g., RS102895, and RS504393). In some embodiments, the class II MMI compound of Formula (I) is a compound of Formula (II), Formula (III), Formula (IV), or Formula (V). In some embodiments, the method further comprises administering, in combination with the one or more MMIs, another agent, e.g., that augments the MMIs ability to inhibit tumor growth or metastasis. In some embodiments, the method comprises administering, in combination with the one or more MMIs, a receptor tyrosine kinase inhibitor (TKI). In some embodiments, the TKI is sunitinib. In some embodiments, administration of the TKI in combination with one or more MMIs results in an additive increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with a TKI or a MMI alone. In some embodiments, administration of the TKI in combination with one or more MMIs results in a synergistic increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with a TKI or a MMI alone.
In some embodiments, the method comprises administering, in combination with the one or more MMIs, a non-specific immunostimulant (e.g., CLDC). In certain embodiments, an MMI, a TKI, or a combination of a MMI and a TKI is administered to an individual. In certain embodiments, MMI, a TKI, and a non-specific immunostimulant is administered to an individual.

In certain embodiments, the cancer is an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.

In certain embodiments, the compounds of Formula (I) described herein (e.g., Ondansetron or Alosetron) and the ARBs of the present invention are used to inhibit monocyte migration, thus amplifying cancer vaccine immunity by enhancing an immune response. These uses/methods include administering an effective amount of an ARB or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron) in conjunction with an anti-tumor preparation to an individual. In some embodiments, the ARB or the compound of Formula (I) (e.g., Ondansetron and/or Alosetron) is administered in conjunction with an anti-tumor preparation to inhibit the tumor growth or metastasis. In particular embodiments, the tumor is epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor. In some embodiments, the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, is administered in conjunction with an anti-tumor preparation to inhibit the tumor growth or metastasis, e.g., of an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.

Additionally, inhibition of monocyte migration into tumor tissues can suppress monocyte pro-metastatic actions (such as monocyte-induced establishing of the metastatic niche), thus in some embodiments, the present invention provides for methods of suppressing tumor growth or metastasis directly by modulating monocyte migration. Accordingly, in
some instances, one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof is administered to an individual with cancer to suppress tumor growth or metastasis. The one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof can be administered as an anti-tumor growth and/or anti-metastatic monotherapy, or the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof can be administered in conjunction with an anti-tumor preparation to inhibit the tumor growth or metastasis of an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.

In some embodiments, tumor growth and/or metastasis in an individual to whom one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof is administered in conjunction with an anti-tumor preparation is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 25%, at least about 30%>, at least about 40%>, at least about 50%>, at least about 60%>, at least about 70%>, at least about 75%, at least about 80%>, at least about 85%, at least about 90%, or at least about 95% as compared to tumor growth in an individual who receive the anti-tumor preparation alone.

In some embodiments, tumor growth and/or metastasis in an individual to whom one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof is administered as a monotherapy is reduced by at least about 5%, at least about 10%>, at least about 20%>, at least about 25%>, at least about 30%>, at least about 40%>, at least about 50%>, at least about 60%>, at least about 70%>, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% as compared to tumor growth in an individual who do not receive the monocyte migration inhibitor(s).

In some embodiments, the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or
combination thereof administered as a monotherapy (or in combination with or in conjunction with the anti-tumor preparation) can be any compound, combination of compounds, or any prodrug, salt, or derivative of compound of an MMI such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) disclosed herein. In some embodiments, the anti-tumor preparation can be administered in conjunction with Alosetron. In some embodiments, the anti-tumor preparation can be administered in conjunction with Ondansetron. In some embodiments the anti-tumor preparation can be administered in conjunction with an ARB, e.g., Losartan. The ARB administered in conjunction with the anti-tumor preparation can be any ARB, combination of ARBs, or any prodrug, salt, or derivative of an ARB described herein. The ARB or the compound of Formula (I) and an anti-tumor preparation can be present in a single pharmaceutical composition, or they can be provided in separate compositions that can be administered in any order relative to one another or administered simultaneously, as described herein. Compositions containing the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron)), or combination thereof and/or the anti-tumor preparation can be administered according to any method known in the art at dosages described elsewhere herein.

The individuals to whom a compound of Formula (I) is administered in conjunction with the anti-tumor preparation can be those who are otherwise not receiving Ondansetron or Alosetron for the treatment of, e.g., irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), chemotherapy-induced nausea and vomiting (CINV), radiation-induced nausea and vomiting (RINV), and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or is otherwise in need of treatment, can receive Ondansetron and/or Alosetron in conjunction with an anti-tumor preparation for the purpose of enhancing an immune response if the individual is not being treated with Ondansetron and/or Alosetron. Alternatively, in certain embodiments, the individuals on whom the methods are practiced have temporarily suspended Ondansetron and/or Alosetron treatment and have been shown, using methods well known in the art, to not have detectable levels of Ondansetron and/or Alosetron in their blood and/or urine prior
to the administration of the Ondansetron and/or Alosetron in conjunction with the anti-tumor preparation.

In certain embodiments, the individuals to whom an ARB is administered in conjunction with an anti-tumor preparation can be those who are otherwise not receiving an ARB for treatment of a pre-existing condition, e.g., hypertension, diabetic nephropathy, heart failure, and/or other conditions, as described herein. In some embodiments, an individual who has a condition or disease described herein, or who is otherwise in need of treatment, can receive an ARB in conjunction with an anti-tumor preparation for the purpose of enhancing an immune response if the individual is not being treated with an ARB.

Alternatively, in certain embodiments, the individuals to whom an ARB is administered in conjunction with an anti-tumor preparation can be those who have temporarily suspended ARB treatment. For example, an individual who is being treated with an ARB for a pre-existing condition can receive an ARB in conjunction with an anti-tumor preparation if the individual has taken the ARB for treatment more than about 10 minutes, more than about 30 minutes, more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than about 24 hours, more than about 1 day more than about 2 days, more than about 3 days, more than about 4 days, more than about 5 days, more than about 6 days, more than about 1 week, more than about 3 weeks, more than about 1 month, more than about 2 months, more than about 3 months, more than about 4 months, more than about 5 months, more than about 6 months, more than about 7 months, more than about 8 months, more than about 9 months, more than about 10 months, more than about 11 months, or more than about 1 year before receiving the ARB in conjunction with the anti-tumor preparation, including any range in between these values.

Alternatively, an individual who is being treated with an ARB for a pre-existing condition can suspend ARB treatment and be tested, using methods well known in the art, to determine that the individual does not have a detectable level of an ARB in their blood and/or urine prior to the administration of the ARB in conjunction with the anti-tumor preparation.

In some embodiments, the method of suppressing tumor growth or metastasis in an individual with cancer comprises administering to the individual two or more MMIs. In certain embodiments at least two MMIs having different mechanisms of action for inhibiting
monocyte migration are administered to the individual to suppress tumor growth or metastasis. In some preferred embodiments, two MMIs having different mechanisms of action for inhibiting monocyte migration are administered to the individual in conjunction with a TKI to suppress tumor growth or metastasis.

In one embodiment, a Class I MMI, as described herein and a Class II MMI, as described herein are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class I MMI, as described herein and a Class III MMI, as described herein are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class II MMI, as described herein and a Class III MMI, as described herein are administered in combination to suppress tumor growth or metastasis in an individual with cancer.

In one embodiment, a Class I MMI, as described herein, a Class II MMI, as described herein, and a TKI, e.g., sunitinib, are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class I MMI, as described herein, a Class III MMI, as described herein, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one embodiment, a Class II MMI, as described herein, a Class III MMI, as described herein, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer.

In one certain embodiment ondansetron, irbesartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, ondansetron, telmisartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, ondansetron, Losartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, irbesartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, telmisartan, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, Losartan, and sunitinib are administered in combination to suppress
tumor growth or metastasis in an individual with cancer. In one certain embodiment, RSI 02895, ondansetron, and sunitinib are administered in combination to suppress tumor growth or metastasis in an individual with cancer.

### Anti-Tumor Preparations

The anti-tumor preparation can be any anti-tumor preparation known and used in the art. For example, certain anti-tumor preparations inhibit the synthesis of new DNA strands, thus preventing tumor cells from replicating. In certain embodiments, the anti-tumor preparation can be an antimetabolite, such as 5-fluorouracil, methotrexate, capecitabine, Alimta, gemcitabine, etc. In certain embodiments, the anti-tumor preparation can be a platinum-based agent, such as cisplatin, carboplatin, oxaliplatin, and the like, which cross-link DNA and inhibit DNA repair in tumor cells. In certain embodiments, the anti-tumor preparation can be an alkylating agent, such as cyclophosphamide, carmustine (BCNU), methyl-CCNU, or piposulfan. In certain embodiments, the anti-tumor agent can be a tyrosine kinase inhibitor, such as gefitinib (Iressa®), imatinib (Gleevec®), lapatinib, sunitinib (Sutent®), GW2580, or Tarceva. In certain embodiments, the anti-tumor preparation can be an anthracycline, such as actinomycin, doxil, doxorubicin (adriamycin), epirubicin, or mitoxantrone. In certain embodiments, the anti-tumor preparation can be a topoisomerase inhibitor, such as camptothecin, irinotecan, topotecan, etoposide, amsacrine, etoposide phosphate, or teniposide.

Other anti-tumor preparations interfere with microtubule assembly or disassembly, thus interrupting tumor cell division. In certain embodiments, the anti-tumor preparation can be a vinca alkaloid, such as vinblastine, vincristine, vindesine, VP-16, or vinorelbine (Navelbine®). Other anti-tumor agents that exhibit anti-microtubule activity include colchicine, taxanes and taxane derivatives. Additional anti-tumor preparations include proteasome inhibitors (e.g., bortezomib (Velcade)); anti-angiogenesis agents; and therapeutic antibodies (e.g., anti-VEGF antibody/Avastin®/bevacizumab, anti-HER2 antibody/Herceptin®/trastuzumab, Erbitux®/cetuximab, Campath/Alemtuzumab, Myelotarg/gemtuzumab, Zevalin/ibritumomab tiuxetan, Rituxan/rituximab, and Bexxar/tositumomab). In certain embodiments, two or more anti-tumor preparations can be
administered in combination with (e.g., in conjunction with) one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combinations thereof.

In certain embodiments, the anti-tumor preparation that is administered in combination with (e.g., in conjunction with) one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof can be a cancer vaccine, e.g., a cancer vaccine designed to increase the targeted immune response against cancer cells already present in the individual. In certain embodiments, the cancer vaccine can contain whole inactivated tumor cells, parts of tumor cells, or tumor cell lysates. In certain embodiments, the cancer vaccine includes a tumor-specific antigen, such as a protein, peptide, or carbohydrate. In certain embodiments, the cancer vaccine that is administered in combination with (e.g., in conjunction with) one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof can include a nucleic acid, viral vector, or bacterial vector that encodes a tumor-specific antigen. In certain embodiments, the cancer vaccine can be a dendritic cell vaccine that has been pulsed with a tumor-specific antigen. The tumor-specific antigen can be any tumor-specific antigen known in the art, including, but not limited to, e.g., melanA/MART-, NY-ESO-1, MAGE, ETA, CEA, CA-125, CA15-3, CA27-29, CA19-9, MUC-1, AFP, an abnormal product of the ras gene, an abnormal product of the p53 gene, CAMEL, Ep-CAM, her-2/neu, WT-1, EBNA3, CD10, CD34, CD99, CD1 17, CD45 (PTPRC), chromogranin, mucin, LMP2, E6, E7, K12, K8.1, tyrosinase, proteinase 3, GM2, GD2, GD3, polysialic acid, fucosyl GM1, globo H, KSA, sialyl Leα, Leβ, TF, Tn, sTn, PSMA, PSA, MUC16, SAGE1, HBA-71, calretinin, carcinoembryonic antigen, a cytokeratin, desmin, EMA, Factor VIII, CD31 FL1, GFAP, GCDFP-15, HMB-25, an immunoglobulin, inhibin, keratin, a lymphocyte marker, Myo Dl, MSA, neurofilament, NSE, PLAP, S100, SMA, synaptophysin, thyroglobulin, vimentin, tumor M2-PK, thyroid transcription factor-1, a squamous cell carcinoma tumor-specific antigen, an osteosarcoma tumor-specific antigen, or a brain tumor-specific antigen. Alternatively, the cancer vaccine can include a peptide or polysaccharide derived from any of the tumor-specific antigen listed above.
Administering an ARB or a compound of Formula (I) in Conjunction with an
Antigen, Vaccine, or Anti-Tumor Preparation

Various methods described above entail administering one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, in combination with (e.g., in conjunction with) an antigen, a vaccine, or an anti-tumor preparation. The one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, and the antigen, vaccine, or anti-tumor preparation can be present in a single composition. Alternatively, the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, and the antigen, vaccine, or anti-tumor preparation can be provided in separate compositions. For example, the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, can be provided as a tablet for oral administration, and the antigen, vaccine, or anti-tumor preparation can be provided in an injectable composition. Where the the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, and the antigen, vaccine, or anti-tumor preparations are present in separate compositions, the two or more compositions can be administered in any order relative to one another. For example, in certain embodiments wherein two separate compositions are administered, one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof, can be administered as a single composition before the antigen, vaccine, or anti-tumor preparation. Alternatively, the antigen, vaccine, or anti-tumor preparation can be administered before the composition comprising the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof. Of course, the skilled artisan will appreciate that each of the individual compositions that are administered to the individual may be administered in any sequencial order. The pharmaceutical composition containing the one or more MMIs such as, e.g., an ARB, a CCR2 antagonist, or a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), or combination thereof,
and the pharmaceutical composition containing the antigen, vaccine, or anti-tumor preparation can be administered within at least about 12 hours, within at least about 11 hours, within at least about 10 hours, within at least about 9 hours, within at least about 8 hours, within at least about 7 hours, within at least about 6 hours, within at least about 5 hours, within at least about 4 hours, within at least about 3 hours, within at least about 2 hours, within at least about 1 hour, or less than 1 hour (e.g., within about 45 minutes, within about 30 minutes, within about 15 minutes, within about 10 minutes, within about 5 minutes, less than 5 minutes, less than 4 minutes, less than 3 minutes, less than 2 minutes, or less than 1 minute) of one another, including any ranges between these values. In certain embodiments, the two compositions can be administered simultaneously, e.g., premixed and administered.

Accordingly, in certain embodiments, the compositions of the invention include Ondansetron and an antigen, vaccine, or anti-tumor preparation. In certain embodiments, the composition is administered orally. In certain embodiments, Ondansetron in the orally administered composition is at a concentration sufficient to provide a dose of at least about 0.5 mg, at least about 1 mg, at least about 2 mg, at least about 3 mg, at least about 4 mg, at least about 5 mg, at least about 6 mg, at least about 7 mg, at least about 8 mg, at least about 10 mg, or less than about 12 mg, or less than about 18 mg, or less than about 24 mg of Ondansetron, including any range in between these values. In certain embodiments, the Ondansetron in the orally administered composition is at a concentration sufficient to provide a dose of more than about 24 mg, more than about 26 mg, more than about 28 mg, more than about 30 mg, more than about 32 mg, or more than about 34 mg Ondansetron, including any range in between these values. In certain embodiments, compositions are administered intravenously. Compositions for intravenous administration can include Ondansetron at a concentration sufficient to provide a dose of at least about 0.01 mg/kg/day, at least about 0.05 mg/kg/day, at least about 0.1 mg/kg/day, at least about 0.15 mg/kg/day, at least about 0.2 mg/kg/day, at least about 0.3 mg/kg/day, at least about 0.4 mg/kg/day, at least about 0.5 mg/kg/day, at least about 0.75 mg/kg/day, at least about 1.0 mg/kg/day, at least about 1.5 mg/kg/day, at least about 2.0 mg/kg/day, at least about 2.5 mg/kg/day, at least about 3.0 mg/kg/day, at least about 3.5 mg/kg/day, at least about 4.0 mg/kg/day, at least about 4.5 mg/kg/day, at least about 5.0 mg/kg/day, at least about 5.5 mg/kg/day, at least
about 6.0 mg/kg/day, at least about 6.5 mg/kg/day, at least about 7.0 mg/kg/day, at least about 7.5 mg/kg/day, at least about 8.0 mg/kg/day, or less than about 8.5 mg/kg/day of Ondansetron, including any range between these values.

For example, the invention provides compositions that can include Alosetron and an antigen, vaccine, or anti-tumor preparation, where the Alosetron in the composition is at a concentration sufficient to provide a dose of at least about 0.1 mg, at least about 0.2 mg, at least about 0.3 mg, at least about 0.4 mg, or less than about 0.5 mg Alosetron, including any range between these values. In certain embodiments, the Alosetron in the composition is at a concentration sufficient to provide a dose of more than about 2 mg, more than about 3 mg, more than about 4 mg, more than about 5 mg, or more than about 6 mg Alosetron, including any range in between these values.

The ARB administered in conjunction with the antigen, vaccine, or anti-tumor preparation can be any ARB, combination of ARBs, or any prodrug, salt, or derivative of an ARB described herein. For example, Losartan can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation at a concentration sufficient to provide a dose of at least about 5 mg, at least about 10 mg, at least about 15 mg, at least about 20 mg, or less than about 25 mg of Losartan, including any range in between these values. In certain embodiments, the Losartan in the composition is at a concentration sufficient to provide a dose of more than about 80 mg, more than about 100 mg, more than about 125 mg, more than about 150 mg, more than about 175 mg, or more than about 200 mg or Losartan, including any range in between about 5 mg and about 200 mg.

In certain embodiments, compositions of the invention can include Losartan at a concentration sufficient to provide a dose of at least about 0.5 mg/kg, at least about 0.75 mg/kg, at least about 1.0 mg/kg, at least about 1.25 mg/kg, at least about 1.5 mg/kg, at least about 1.75 mg/kg, or at least about 2.0 mg/kg of Losartan, including any range between about 0.5 mg/kg and about 1.75 mg/kg. In certain embodiments, compositions of the invention can include Losartan at a concentration sufficient to provide a dose of more than about 1.75 mg/kg, at least about 2.0 mg/kg, at least about 5 mg/kg, at least about 7 mg/kg, at least about 10 mg/kg, at least about 12 mg/kg, at least about 15 mg/kg, at least about 17 mg/kg, at least about 20 mg/kg, at least about 22 mg/kg, at least about 25 mg/kg, at least
about 27 mg/kg, or at least about 30 mg/kg or Losartan, including any range in between about 1.75 mg/kg and about 30 mg/kg. In certain embodiments, the compositions of the invention can include Losartan at a concentration sufficient to provide a dose of more than about 30 mg/kg, e.g., at least about 35 mg/kg or at least about 40 mg/kg of Losartan, including any range in between about 30 mg/kg and about 40 mg/kg.

In certain embodiments, Candesartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 4 mg, less than about 3 mg, less than about 2 mg, or less than about 1 mg, including any range between these values. In certain embodiments, Candesartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 32 mg, more than about 40 mg, more than about 48 mg, more than about 56 mg, or more than about 64 mg, including any range in between these values.

Eprosartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 400 mg, less than about 300 mg, less than about 200 mg, less than about 100 mg, or less than about 50 mg, including any range between these values. In certain embodiments, Eprosartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 600 mg, more than about 750 mg, more than about 900 mg, more than about 1050 mg, or more than about 1200 mg, including any range in between these values.

Irbesartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of about less than 150 mg, less than about 100 mg, less than about 50 mg, or less than about 25 mg, including any range between these values. In certain embodiments, Irbesartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 300 mg, more than about 375 mg, more than about 450 mg, more than about 525 mg, or more than about 600 mg, including any range in between these values.

Olmesartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 20 mg, less than about 15 mg, less than about 10 mg, or less than about 5 mg, including any range between these values. In certain embodiments, Olmesartan can be administered in conjunction with an antigen, vaccine, or
anti-tumor preparation at a dosage of more than about 40 mg, more than about 50 mg, more than about 60 mg, more than about 70 mg, or more than about 40 mg, including any range in between these values.

Telmisartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 20 mg, less than about 15 mg, less than about 10 mg, or less than about 5 mg, including any range between these values. In certain embodiments, Telmisartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 80 mg, more than about 100 mg, more than about 120 mg, more than about 140 mg, or more than about 160 mg, including any range in between these values.

Valsartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 20 mg, less than about 15 mg, less than about 10 mg, or less than about 5 mg, including any range between these values. In certain embodiments, Valsartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 320 mg, more than about 400 mg, more than about 480 mg, more than about 560 mg, or more than about 640 mg, including any range in between these values.

Azilsartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of less than about 40 mg, less than about 30 mg, less than about 20 mg, or less than about 10 mg, including any range between these values. In certain embodiments, Azilsartan can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation at a dosage of more than about 80 mg, more than about 100 mg, more than about 120 mg, more than about 140 mg, or more than about 160 mg, including any range in between these values.

METHOD OF USING TWO OR MORE MONOCYTE MIGRATION INHIBITORS

The methods described herein all relate to the use of various monocyte migration inhibitors for effecting treatment on individuals. Any and all of the embodiments disclosed in the present specification with regard to the use of a single monocyte migration inhibitor for the aforementioned methods, are also meant to encompass the same methods wherein two
or more monocyte migration inhibitors are administered together, or used in conjunction with one another (collectively "combined") according to the various methods. In this context (i.e., in reference to the administration to two or more monocyte migration inhibitors) "administered together" encompasses administering a first MMI before a second MMI, administering the first MMI after the second MMI, administering the first MMI in conjunction with the second MMI, and administering the first MMI simultaneous to the second MMI (e.g., as a single composition comprising the first and second MMIs). Thus, without wishing to be limited by example, the present invention provides for the use of two or more monocyte migration inhibitors for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

As disclosed in this specification, the methods of the present invention may optionally be carried out by also administering an antigen, vaccine, or anti-tumor preparation in before, after, in conjunction with, or simultaneously (e.g., as a single composition) with the two or more monocyte migration inhibitors, however in some instances the two or more monocyte migration inhibitors may also be administered in the absence of these.

Any two or more monocyte migration inhibitors may be combined for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual. In certain embodiments, at least one of the two or more MMIs is described herein.

In some instances, the MMIs that are combined for administration to the individual are selected from Class I MMIs which have angiotensin receptor blocking activity, Class II MMIs which have serotonin receptor antagonist activity, and Class III MMIs which have CCR2 receptor antagonist activity.

Accordingly, non-limiting examples of Class I MMIs include but are not limited to Azilsartan, Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, and Valsartan;

Non-limiting examples of Class II MMIs include but are not limited to Ondansetron, and related MMI compounds of Formula (I):
wherein

$R^1$ is hydrogen or C$_6$H$_{13}$ alkyl;

$Q^1$ is a single bond or double bond;

$R^2$ is selected from hydrogen and C$_6$H$_{13}$ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO$_2$-, -NR$_a$-, or -CO-; wherein $R^a$ is hydrogen or C$_6$H$_{13}$ alkyl; and

$R^3$ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C$_6$H$_{13}$ alkyl;

or pharmaceutically acceptable salts thereof;

In some embodiments, the Class II MMI of Formula (I) is a compound of Formula (II), Formula (III), Formula (IV), or Formula (V).

Non-limiting examples of Class III MMIs include but are not limited to antagonists of the CCR2 receptor, e.g., the competitive antagonists RS102895, RS504393, and related compounds e.g., disclosed in Higgins et al. (2007) (Chemokine Biology-Basic Research an Clinical Application, Vol. II, Birkhauser Verlag Basel Switzerland, pg. 115-123), and Mirzadegan et al. (2000)(The Journal of Biological Chemistry, Vol. 275, No.33, Aug, pg. 25562-25571), incorporated herein in their entirety.

In some instances, it is preferable to combine two or more MMI's having different mechanisms of action for inhibiting monocyte migration, as such a combination may in some cases result in a synergistic inhibition of monocyte migration. Accordingly, e.g., two or more inhibitors from different classes listed above may be combined for use in the methods of the present disclosure for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

In some embodiments, administration of a Class I MMI in combination with a Class
II MMI, e.g., in conjunction with an antigen, vaccine, or anti-tumor preparation, results in synergistic inhibition of monocyte migration. For example, administration of ondansetron combined with irbesartan results in a synergistic inhibition of monocyte migration inhibition; administration of ondansetron combined with telmisartan results in a synergistic inhibition of monocyte migration inhibition; and administration of ondansetron combined with Losartan results in a synergistic inhibition of monocyte migration inhibition. In particular embodiments, the present invention contemplates each of these combinations of MMIs.

In some instances, administration of a Class I MMI in combination with a Class III MMI, e.g., in conjunction with an antigen, vaccine, or anti-tumor preparation, results in synergistic inhibition of monocyte migration. For example, administration of RSI 02895 combined with irbesartan results in a synergistic inhibition of monocyte migration inhibition; administration of RSI 02895 combined with telmisartan results in a synergistic inhibition of monocyte migration inhibition, and administration of RSI 02895 combined with Losartan results in a synergistic inhibition of monocyte migration inhibition. In particular embodiments, the present invention contemplates each of these combinations of MMIs.

Non-limiting examples of the MMI combinations that the present invention contemplates include the following embodiments:

(i) In certain embodiments, Losartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Losartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Losartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Losartan and RS504393 are combined together or used in conjunction with one another.

(j) In certain embodiments, Candesartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Candesartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Candesartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Candesartan and RS504393 are combined together or used in conjunction with one another.
(k) In certain embodiments, Eprosartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Eprosartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Eprosartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Eprosartan and RS504393 are combined together or used in conjunction with one another.

(1) In certain embodiments, Irbesartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Irbesartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Irbesartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Irbesartan and RS504393 are combined together or used in conjunction with one another.

(m) In certain embodiments, Olmesartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Olmesartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Olmesartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Olmesartan and RS504393 are combined together or used in conjunction with one another.

(n) In certain embodiments, Telmisartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Telmisartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Telmisartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Telmisartan and RS504393 are combined together or used in conjunction with one another.

(o) In certain embodiments, Azilsartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Azilsartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Azilsartan and RSI 02895 are combined together or used in conjunction with one another. In certain embodiments, Azilsartan and RS504393 are combined together or used in conjunction with one another.
(p) In certain embodiments, Valsartan and Ondansetron are combined together or used in conjunction with one another. In certain embodiments, Valsartan and Alosetron are combined together or used in conjunction with one another. In certain embodiments, Valsartan and RS1 02895 are combined together or used in conjunction with one another. In certain embodiments, Valsartan and RS504393 are combined together or used in conjunction with one another.

In some embodiments, any MMI (e.g., the MMIs described herein), or any MMI combination described herein, (e.g., any one of the non-limiting examples of the MMI combinations presented in (i) - (p) above) is combined with one or more general non-specific immunostimulants (e.g., a cationic lipid DNA complex or "CLDC"). In some embodiments, one or more MMIs and one or more general immunostimulants are comprised in a single composition. In some embodiments, the one or more MMIs and the one or more general immunostimulants are comprised in separate compositions. In some embodiments a composition comprising one or more immunostimulant is administered to an individual before, after, or in conjunction a composition comprising one or more MMIs, according to the present disclosure. All such compositions are contemplated for use in the methods of the present disclosure, e.g., for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

In still further embodiments, any MMI (e.g., the MMIs described herein), or any MMI combination described herein, (e.g., any one of the non-limiting examples of the MMI combinations presented in (i) - (p) above), optionally combined with one or more general immunostimulants (e.g., CLDC), may also be combined with one or more receptor tyrosine kinase inhibitor (TKI), e.g., sunitinib. In some embodiments, combinations of one or more MMI with one or more TKI can result in an additive increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with the TKIs or the MMIs alone. Furthermore, in some instances, administration of the one or more TKI in combination with the one or more MMI results in a synergistic increase in the suppression of tumor growth or metastasis as compared to the suppression that would be achieved by treating with the one or more TKIs or one or more MMIs alone.
Particular embodiments of the present invention contemplate the combination of sunitinib with any of the MMIs or MMI combinations of the present invention (e.g., any one of the non-limiting examples of the MMI combinations presented in (i) - (p) above). Still further embodiments contemplate such combinations further combined with CLDC. In some embodiments, the one or more MMIs and the one or more TKIs (e.g., sunitinib) are comprised in the same composition. In some embodiments, the one or more MMIs and the one or more TKIs (e.g., sunitinib) are comprised in separate compositions. In some embodiments a composition comprising one or more TKI is administered to an individual before, after, or in conjunction a composition comprising one or more MMIs, according to the present disclosure. In certain such embodiments, such compositions are combined with general immunostimulants (e.g., CLDC) optionally by adding the immunostimulant to any one of the compositions described above, or by administering the immunostimulant before, after, or in conjunction with any one of the compositions described above. All such compositions are contemplated for use in the methods of the present disclosure, e.g., for inhibiting the migration of monocytes, for enhancing an immune response, for decreasing monocyte migration to a lymph node, for amplifying vaccine immunity, and for reducing tumor growth or metastasis in an individual.

In certain embodiments, cancers treated in this manner (i.e., by administering two or more combined MMIs, optionally combined with a TKI, and also optionally combined with an immunostimulant) are selected from an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor. Accordingly, in some embodiments, the method of suppressing tumor growth or metastasis in an individual with cancer comprises administering to the individual two or more MMIs. In certain embodiments at least two MMIs having different mechanisms of action for inhibiting monocyte migration are administered to the individual to suppress tumor growth or metastasis. In some preferred embodiments, two MMIs having different mechanisms of action for inhibiting monocyte migration are administered to the individual in conjunction with a TKI to suppress tumor growth or metastasis.
**Modes of Administration**

Administration of any one of the compositions described herein, e.g., one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RSI 02895 or RS504393), and optionally combined with an antigen, vaccine, or anti-tumor preparation, and/or optionally combined with a receptor tyrosine kinase inhibitor (e.g., sunitinib), and/or optionally a general immunostimulant (e.g., CLDC) (collectively "The Composition") can be achieved by a variety of routes, including, e.g., topical application, inhalation, intravenous injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraventricularly, intra-articularly, intraocularly, intraspinally, or by other methods well known to those of skill in the art.

The one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RSI 02895 or RS504393) and the antigen, vaccine, or anti-tumor preparation and/or the optional TKI and/or general immunostimulant can be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation. When one or more MMIs and the antigen, vaccine, or anti-tumor preparation are present in two or more separate pharmaceutical compositions, each composition can optionally be administered through a different route. In some embodiments, the one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RSI 02895 or RS504393) are administered in conjunction with the antigen, vaccine, or anti-tumor preparation using any medically appropriate procedure, e.g. intravascular (intravenous, intraarterial, intracapillary) administration, injection into the cerebrospinal fluid, intracavity or direct injection in the brain. Intrathecal administration may be carried out through the use
of an Ommaya reservoir, in accordance with known techniques. (F. Balis et al., Am J. Pediatr. Hematol. Oncol. 11, 74, 76 (1989). In some such embodiments, a TKI and/or a general immunostimulant is further administered in conjunction with the aforementioned MMIs and the antigen, vaccine, or anti-tumor preparation using any medically appropriate procedure, e.g. intravascular (intravenous, intraarterial, intracapillary) administration, injection into the cerebrospinal fluid, intracavity or direct injection in the brain. In some such embodiment, a TKI and/or a general immunostimulant is further administered before, after, or simultaneous to the aforementioned MMIs or the antigen, vaccine, or anti-tumor preparation using any medically appropriate procedure, e.g. intravascular (intravenous, intraarterial, intracapillary) administration, injection into the cerebrospinal fluid, intracavity or direct injection in the brain.

In some embodiments, the one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RSI 02895 or RS504393) can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation prior to exposure of the individual to, e.g., an infectious agent, so that the resulting immune response upon subsequent exposure to the infectious agent can reduce the severity and/or duration of, e.g., the infection or disease caused by the infectious agent. An effective amount of such MMIs or MMI combinations can be provided in one or more administrations. For example, where the antigen, vaccine, or anti-tumor preparation and such MMIs or MMI combinations are provided in separate pharmaceutical compositions, an individual can receive the antigen, vaccine, or anti-tumor preparation and effective amount of the MMIs or MMI combinations, and then receive additional administrations of the MMIs or MMI combinations 12 hours, 24 hours, 36 hours, 48 hours, and/or more than 48 hours after the initial administration.

For therapeutic treatment, the one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RS102895 or RS504393) can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation to an individual who is
already exposed to the pathogen or has the infection or disease, e.g., cancer. The resulting enhanced immune response can reduce the duration and/or severity of the existing diseases or infection, as well as minimize any harmful consequences of untreated disease or infection. The one or more MMIs singularly or in combination selected from an ARB (e.g., Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan), a compound of Formula (I) (e.g., Ondansetron and/or Alosetron), and a CCR2 antagonist (e.g., RSI 02895 or RS504393) can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation along with any other therapeutic regimen.

**Individuals Who Can Be Treated Using the Methods**

Various individuals can be treated using the methods described herein. In certain embodiments, the individual who can be treated using the methods described above can be a patient.

In certain embodiments, an ARB (e.g., Losartan) or a combination of ARBs can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation to individuals who are otherwise not receiving an ARB for treatment of a pre-existing condition. As noted elsewhere herein, ARBs can be prescribed for the treatment and/or prevention of a variety of diseases. Accordingly, it will be appreciated by one of skill in the art that an individual on whom any method of the invention is practiced does not have, e.g., congestive heart failure, chronic heart failure, hypertension, diabetic neuropathy, migraine, a predisposition to myocardial infarction, or any other condition for which ARBs are indicated, or is not otherwise in need of treatment. An individual who has a condition or disease described herein can receive an ARB in conjunction with an antigen for the purpose of enhancing an immune response if the individual is not being treated with an ARB.

In certain embodiments, a compound of Formula (I) (e.g., Ondansetron or Alosetron), or a combination of compounds of Formula (I) can be administered in conjunction with the antigen, vaccine, or anti-tumor preparation. A compound or combination of compounds of Formula (I) (e.g., Ondansetron and/or Alosetron) can be administered in conjunction with an antigen, vaccine, or anti-tumor preparation to individuals who are otherwise not receiving Ondansetron and/or Alosetron for treatment of a pre-existing condition. As noted elsewhere herein, Ondansetron and/or Alosetron can be prescribed for the treatment and/or prevention
of a variety of diseases. Accordingly, it will be appreciated by one of skill in the art that an individual on whom any method of the invention is practiced does not have, e.g., irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONV), radiation-induced nausea and vomiting (RINV), chemotherapy-induced nausea and vomiting (CINV), or is not otherwise in need of Ondansetron treatment and/or Alosetron treatment. An individual who has a condition or disease described herein, or is otherwise in need of treatment, can receive Ondansetron, Alosetron in conjunction with an antigen, vaccine, or anti-tumor preparation for the purpose of enhancing an immune response if the individual is not being treated with Ondansetron and/or Alosetron.

Alternatively, in some embodiments the individuals who are being treated with an ARB or with Ondansetron and/or Alosetron can temporarily suspend the ARB or Ondansetron and/or Alosetron treatment. For example, an individual who is being treated with Ondansetron and/or Alosetron can receive Ondansetron, Alosetron, and/or a combination of compounds of Formula (I) comprising Ondansetron and/or Alosetron in conjunction with an antigen, vaccine, or anti-tumor preparation if the individual has taken Ondansetron and/or Alosetron more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than 24 hours, more than 1 day more than 2 days, more than 3 days, more than 4 days, more than 5 days, more than 6 days, more than 1 week, more than 3 weeks, more than 1 month, more than three months, more than 6 months, or more than 1 year before receiving Ondansetron, Alosetron, and/or a combination of compounds of Formula (I) comprising Ondansetron and/or Alosetron in conjunction with the antigen, including any range in between these values.

Similarly, in some embodiments, the individuals who are being treated with an ARB can temporarily suspend ARB treatment. For example, an individual who is being treated with an ARB for a pre-existing condition can receive an ARB in conjunction with an antigen, vaccine, or anti-tumor preparation if the individual has taken the ARB more than about 1 hour, more than about 3 hours, more than about 6 hours, more than about 12 hours, more than about 18 hours, more than 24 hours, more than 1 day more than 2 days, more than 3 days, more than 4 days, more than 5 days, more than 6 days, more than 1 week, more than 3 weeks, more than 1 month, more than three months, more than 6 months, or more than 1 year.
before receiving the ARB in conjunction with the antigen, including any range in between these values.

Alternatively, in some embodiments, individuals receiving an ARB or Ondansetron and/or Alosetron therapy can temporarily suspend treatment prior to being administered the ARB or Ondansetron, Alosetron, and/or a combination compounds of Formula (I) comprising Ondansetron and/or Alosetron in conjunction with an antigen, vaccine, or anti-tumor preparation for the purpose of enhancing an immune response, inhibiting monocyte migration to a lymph node, amplifying vaccine immunity, or treating cancer. For example, blood and/or urine samples provided by such an individual can be assayed for the presence of the ARB or Ondansetron and/or Alosetron (and/or its metabolites) using chromatographic or spectroscopic methods described elsewhere herein. The methods of the invention can be practiced on the individual once it has been determined that the ARB or Ondansetron and/or Alosetron (and/or its metabolites) is undetectable in the individual's blood and/or urine. Such methods are described elsewhere herein.

EXAMPLES

The following examples are offered to illustrate, but not to limit the claimed invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

EXAMPLE 1

LOSARTAN BLOCKED CANINE AND HUMAN MONOCYTE MIGRATION IN VITRO

Boyden chamber assay.

The Boyden chamber assay is based on a chamber of two medium-filled compartments separated by a microporous membrane. In general, cells are placed in the upper compartment and are allowed to migrate through the pores of the membrane into the lower compartment, in which chemotactic agents are present. After an appropriate incubation
time, the membrane between the two compartments is fixed and stained, and the number of cells that have migrated to the lower side of the membrane is determined. Experiments using this assay were performed as described in Mitchell et al. (2012) Int. Immunopharmacol. 15: 357-363 to assess the migration of monocytes.

A Boyden chamber assay was performed to assess the migration of dog monocytes and of human monocytes (i.e., the human monocyte cell line THP-1) in response to a CCL2 (MCP-1) gradient and to assess the ability of Losartan to inhibit monocyte migration in vitro. As shown in Figure 1, Losartan potently blocked human monocyte migration (THP-1) in vitro in a dose dependent manner at concentrations easily achievable in vivo (hpf= high power field). The asterisks in Figure 1 indicate statistical differences, i.e., a p-value < 0.05. Figure 2 shows that Losartan potently blocked canine monocyte migration in vitro in a dose dependent manner at concentrations easily achievable in vivo. The asterisks in Figure 2 indicate statistical differences, i.e., a p-value < 0.05. Moreover, at concentrations of 1 µg/ml and 10 µg/ml, Losartan blocked canine monocyte migration even in the presence of MCP-1. Taken together, these results indicate that Losartan is an inhibitor of monocyte migration in vitro.

**EXAMPLE 2**

**ONDANSETRON BLOCKED HUMAN MONOCYTE MIGRATION IN VITRO**

A Boyden chamber assay was performed (as described in Mitchell et al. (2012) Int. Immunopharmacol, supra) to assess the migration of human monocytes (i.e., the human monocyte cell line THP-1) in response to a CCL2 (MCP-1) gradient and to assess the ability of Ondansetron to inhibit monocyte migration in vitro. As shown in Figure 3, Ondansetron potently blocked THP-1 monocyte migration in vitro in a dose dependent manner at concentrations easily achievable in vivo. The single asterisk in Figure 3 indicates statistical differences, i.e., a p-value < 0.05. The double asterisks in Figure 3 indicate statistical differences, i.e., a p-value <0.01.
EXAMPLE 3

CCR2 ANTAGONISM BLOCKED MOUSE MONOCYTE MIGRATION IN VITRO

A Boyden chamber assay was performed (as described in Mitchell et al. (2012) Int. Immunopharmacol, *supra*) to assess the migration of mouse peritoneal monocytes in response to a CCL2 (MCP-1) gradient and to assess the ability of RSI 02895 (a CCR2 antagonist) to inhibit monocyte migration in vitro. As shown in Figure 4, RSI 02895 potently blocked monocyte migration in vitro in a dose dependent manner at concentrations easily achievable in vivo. The single asterisk in Figure 4 indicates statistical differences, i.e., a p-value < 0.05.

EXAMPLE 4

ONDANSETRON TREATMENT INHIBITED MONOCYTE MIGRATION TO LYMPH NODES IN MICE

Footpad inflammation-induced monocyte migration assays were performed as previously described (Mitchell et al. (2012), J. Immunology 189: 5612-5621) to determine whether Ondansetron inhibits monocyte migration in vivo in mice. Briefly, two groups of mice (n=4 per group) were injected with 50 µg of liposomal vaccine adjuvant in one footpad. The first group of mice was treated by injection of 3 mg/kg Ondansetron administered IP at the time of footpad injection and the Ondansetron injections were repeated 12, 24, and 36 hours following footpad injection. The second group of mice did not receive any additional treatment. The third group received neither the vaccine nor the Ondansetron. Inflammatory monocyte migration to the draining popliteal lymph nodes was assessed in all three groups of mice via flow cytometry (as described in Mitchell et al. (2012), J. Immunology, *supra*) 24 hours following vaccination. As shown in Figure 5, the migration of inflammatory monocytes to the draining lymph nodes was significantly reduced in mice who received Ondansetron in conjunction with the vaccine as compared to mice who received no additional treatment with vaccination. Figure 6 provides a quantitative analysis of the results depicted in Figure 5. The triple asterisks in Figure 6 indicate statistical differences, i.e., a p-value < 0.001. These results indicate that Ondansetron acts as a potent inhibitor of inflammatory monocyte migration in vivo.
EXAMPLE 5

LOSARTAN TREATMENT INHIBITED MONOCYTE MIGRATION TO LYMPH NODES IN MICE

Footpad inflammation-induced monocyte migration assays were performed as described previously (as described in Mitchell et al. (2012), J. Immunology, supra) to determine whether Losartan inhibits monocyte migration in vivo in mice. Briefly, two groups of mice (n=4 per group) were vaccinated in the right rear footpad. The first group of mice was treated by injection of 30 mg/kg Losartan at the time of injection and 12 hours following injection. The second group of mice did not receive any additional treatment. Inflammatory monocyte migration to the draining popliteal lymph nodes was assessed in all three groups of mice via flow cytometry (as described in Mitchell et al. (2012), J. Immunology, supra) 24 hours following vaccination. As shown in Figure 7, the migration of inflammatory monocytes to the draining lymph nodes was significantly reduced in mice who received Losartan in conjunction with the vaccine as compared to mice who received no additional treatment with vaccination. The asterisks in Figure 7 indicate statistical differences, i.e., a p-value < 0.05. These results indicate that Losartan acts as a potent inhibitor of inflammatory monocyte migration in vivo.

EXAMPLE 6

LOSARTAN TREATMENT INHIBITED MONOCYTE MIGRATION TO LYMPH NODES IN DOGS

To determine whether Losartan inhibits monocyte migration in vivo, dogs [n= 2] were treated with Losartan at 2 mg/kg/day for 48 hours. Blood samples were then taken from the dogs, and Boyden chamber assays were performed as described previously (Mitchell et al. (2012) Int. Immunopharmacol, supra) As shown in Figure 8, monocyte migration was significantly inhibited in blood samples obtained from the dogs after Losartan treatment (8a shows dog 1, 8b shows dog 2, the left panel is a no migration control, the middle panel shows monocyte migration prior to losartan treatment, and the right panel shows monocyte migration after 48 hours of Losartan treatment). The triple asterisks in Figure 8 indicate statistically significant differences (p <0.05). This experiment was repeated in 3 dogs with cancer. Briefly, each of the three dogs was treated for two weeks
with 2 mg/kg/day Losartan before the Boyden chamber assay was performed. Figure 9 provides the results of pooled monocyte migration data (the left panel is a no migration control, the middle panel shows monocyte migration prior to losartan treatment, and the right panel shows monocyte migration after 2 weeks of Losartan treatment). Monocyte migration was inhibited in blood samples obtained from the 3 dogs with cancer who had been receiving Losartan treatment for two weeks. Taken together, these results indicate that Losartan treatment at a concentration of 2 mg/kg/day for at least 48 hours can inhibit monocyte migration in vivo.

**EXAMPLE 7**

**LOSARTAN TREATMENT AT TIME OF VACCINATION SIGNIFICANTLY IMPROVED HUMORAL AND CELLULAR IMMUNITY**

To assess the effects of Losartan on humoral immunity, mice were treated with Losartan at the time of vaccination. Briefly, two groups of mice (n=5) were vaccinated with 5 µg of ovalbumin. The first group of mice received 30 mg/kg Losartan via injection at the time of vaccination, and at 12, 24, and 36 hours following vaccination. The second group of mice received no additional treatment following vaccination. Second vaccinations were repeated 10 days following the initial vaccinations. Second Losartan treatments were repeated for the first group of mice as described. Anti-ovalbumin antibodies from each group of mice were titered (as described in Mitchell et al. (2012), J. Immunology, *supra*) to assess the degree to which humoral immunity was enhanced in the mice receiving Losartan treatment. As shown in Figure 10, mice treated with Losartan were found to have significantly higher anti-ovalbumin antibody titers than mice who received no additional treatment with vaccination, indicating that administration of Losartan in conjunction with the antigen ovalbumin enhanced humoral immunity. The asterisks in Figure 10 indicate statistical differences, i.e., a p-value < 0.05.
EXAMPLE  8
ONDANSETRON TREATMENT AT TIME OF VACCINATION SIGNIFICANTLY
IMPROVED HUMORAL IMMUNITY

To assess the effects of Ondansetron on humoral immunity, mice were treated with
Ondansetron at the time of vaccination. Briefly, two groups of mice (n=4 per group) were
vaccinated subcutaneously with 1 µg of ovalbumin in a liposomal adjuvant. The first group
of mice received 3 mg/kg Ondansetron via i.p. injection at the time of vaccination, and at 12,
24, and 36 hours following vaccination. The second group of mice received no additional
treatment following vaccination. A third group of mice received neither the vaccine nor the
Ondansetron. Second vaccinations were repeated for the first two groups of mice 10 days
following the initial vaccinations, and second Ondansetron treatments were repeated for the
groups as described for the first cycle of vaccination. Anti-ovalbumin antibodies from each
group of mice were titered (as described in Mitchell et al. (2012), J. Immunology, supra) to
assess the degree to which humoral immunity was enhanced in the mice receiving
Ondansetron treatment. As shown in Figure 11, mice treated with Ondansetron were found
to have significantly higher anti-ovalbumin antibody titers than mice who received no
additional treatment with vaccination, indicating that administration of Ondansetron in
conjunction with the antigen ovalbumin enhanced humoral immunity. The asterisk in Figure
11 indicates indicate statistical differences, i.e., a p-value < 0.05.

EXAMPLE  9
LOSARTAN AMPLIFIES VACCINE CELLULAR IMMUNITY

Two groups of mice were immunized as noted in Example 7 above. To determine
whether cellular immunity was enhanced in the Losartan-treated mice, mice from both the
first and second groups were euthanized, and their spleen cells were assayed to determine
IFN-γ secretion levels in response to ovalbumin restimulation. Splenectomies and
restimulation assays for cellular immune responses were performed as previously described
(Mitchell et al. (2012), J. Immunology, supra). Spleen cells were restimulated in vitro for 72
hours with 5 µg/ml ovalbumin protein. As shown in Figure 12, spleen cells from mice that
were vaccinated and treated concurrently with Losartan produced significantly more IFN-γ,
indicating a significant increase in T cell responses to vaccination when losartan was administered in conjunction with the antigen ovalbumin. The asterisks in Figure 12 indicate statistical differences, i.e., a p-value < 0.05.

EXAMPLE 10
ONDANSETRON ADMINISTRATION ENHANCED TUMOR VACCINE RESPONSES

The A20 mouse B-cell lymphoma model was used to assess the effects of Ondansetron on tumor vaccine responses. Briefly, two groups of mice (n = 5 per group) with established d3 A20-HA tumors (i.e., tumors expressing HA) were vaccinated weekly for 3 weeks with HA antigen (1 µg per mouse in liposome adjuvant), either without or with concurrently administered ondansetron (3 mg/kg i.p., administered 4 times at 12 h intervals). A third group of mice received neither the HA nor the Ondansetron. Tumor measurements were made using calipers every 2-3 days. Mice were sacrificed when their tumors reached a diameter of 1.5 cm. As shown in Figure 13, tumor growth rates were significantly reduced in the mice that received the vaccine in conjunction with the Ondansetron, as compared to tumor growth rates in mice that received only the vaccine. The triple asterisks in Figure 13 indicate statistical differences, i.e., a p-value < 0.001. Single asterisks indicate that there are statistically significant differences in tumor sizes between the group of mice who had received Ondansetron and the vaccine and the group of mice that received only the vaccine.

Figure 14 shows tumor tissues from a mouse that received only the vaccine (top panel) and tumor tissues from a mouse that received vaccine in conjunction with Ondansetron (bottom panel) that were evaluated via immunohistochemistry. Labeled anti-CD11b antibodies were used to quantitate the numbers of infiltrating CD11b+ monocyte/macrophages in the tumor tissues. Tumors of mice treated with ondansetron had markedly fewer monocyte/macrophages than tumors of mice that received the vaccine alone. Taken together, the results provided in Figures 13 and 14 demonstrate that administering Ondansetron in conjunction with an anti-tumor vaccine reduced inflammatory monocyte migration into tumor tissues and reduced tumor growth.
EXAMPLE 11

ROUTE OF ADMINISTRATION AND DOSING OPTIMIZATION FOR RSI 02895

To identify optimal routes of administration and dosing schedules for the monocyte migration inhibitor RSI 02895, a preliminary study was conducted wherein RSI 02895 was administered in a variety of ways to mice having tumors metastases. As shown in Figure 15, tumor metastases were established in BALB/c mice by i.v. injection of either $2.5 \times 10^5$ luciferase-expressing CT26 cells or $2.5 \times 10^5$ luciferase-expressing 4T1 cells. One day after tumor cell injection, RSI 02895 was administered to the mice via intraperitoneal injection, subcutaneous injection, or orally via providing mice ad libitum access to water dosed with the drug. Doses and dosing schedules are indicated in the "Route" column of Table 7. Tumor growth was monitored by in vivo imaging (IVIS, Perkin Elmer) which detects the luciferase signal, and was reported as "photon flux." Photon flux signal is proportional to numbers of viable tumor cells and to tumor size. Numbers of monocytes in the lungs was determined by flow cytometry analysis (as described in Example 13). Results of these experiments are summarized in Table 7. These preliminary results demonstrate several suitable routes of administration and dosing schedules.
<table>
<thead>
<tr>
<th>Route</th>
<th>CT26 (colon cancer)</th>
<th>4T1 (breast cancer)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraperitoneal</strong> 5 mg/kg, administered once daily throughout the experiment</td>
<td>Significant decrease in photon counts observed in 1 of 2 experiments. Significant increase in survival of mice in 1 of 2 experiments, significant decrease in monocyte numbers in the lung in 1 of 1 experiment</td>
<td>Significant decrease in photon counts observed in 1 of 1 experiment. Nearly significant decrease in survival of mice in 1 of 1 experiment (p=0.06)</td>
</tr>
<tr>
<td><strong>Intraperitoneal</strong> 5 mg/kg, administered every 12 hours for two days</td>
<td>Significant decrease in photon counts observed in 1 of 1 experiment. No significant increase in survival observed.</td>
<td><strong>ND</strong></td>
</tr>
<tr>
<td><strong>Oral/water</strong> 5 mg/kg/mouse/day based on 3 ml/day/mouse water consumption</td>
<td>Significant decrease in photon counts observed in 1 of 3 experiments. No significant increase in survival observed. No significant changes in monocyte numbers in the lungs. Note: 1 experiment thrown out due to low cell numbers being injected.</td>
<td>Non-significant decrease in photon counts observed in 1 of 1 experiment. Non-significant decrease in monocyte numbers in the lungs (p=0.07) in 1 of 1 experiment. Significant decrease in myeloid derived suppressor cells in the lungs in 1 of 1 experiment.</td>
</tr>
<tr>
<td><strong>Oral/water</strong> 20 mg/kg/mouse/day based on 3 ml/day/mouse water consumption</td>
<td>Non-significant decrease in photon counts observed in 1 of 1 experiment</td>
<td><strong>ND</strong></td>
</tr>
</tbody>
</table>
**EXAMPLE 12**

**TWICE DAILY RSI 02895 ADMINISTRATION VIA Intraperitoneal Injection**

Mice (BALB/c) having syngeneic colon carcinoma cell line CT26-induced metastatic tumors expressing luciferase (established as described in Example 11) were administered RSI 02895 (5mg/kg) twice daily via intraperitoneal injections starting 3 days after i.v. tumor challenge. Tumor growth was monitored by in vivo imaging (IVIS, Perkin Elmer) on days 1, 9, 11, 14, and 16 post tumor injection and is reported as photon flux, which is proportional to tumor size. Figure 16 shows a significant decrease (p<0.01) in photo flux in the mice treated with RSI 02895 as compared to controls (5 per group) indicating that RSI 02895 treatment suppressed tumor growth.

**EXAMPLE 13**

**CCR2 SUPPRESSED GROWTH OF LUNG METASTASES IN MICE**

To assess the ability of CCR2 antagonists to suppress the growth of tumor metastases, BALB/c mice with syngeneic colon carcinoma cell line CT26-induced tumors were used as a model system. As shown in Figure 15, tumor metastases were established in BALB/c mice by i.v. injection of 2.5 X 10^5 luciferase-expressing CT26 cells, and one day after tumor cell injection, once daily treatment with RS504393 (a CCR2 antagonist that is closely related to RSI 02895) was initiated at a dose of 5 mg/kg. Figure 17 shows in vivo
imaging (IVIS, Perkin Elmer) of the luciferase-expressing tumors at day 14 of the experiment. Treatment with RS504393 resulted in a significant inhibition of tumor growth. Moreover, as shown in Figure 18, this treatment resulted in a significant improvement in survival, with one of the five mice having an apparent tumor cure. Thus, we have obtained strong evidence of the efficacy of CCR2 antagonists as a novel class of anti-metastatic drugs.

In a similar experiment, tumor metastases were established in BALB/c mice by i.v. injection of CT26 cells as described above, and once daily treatment with RS504393 was initiated via intraperitoneal administration at 5 mg/kg. On day 14, lung tissues were collected from both the treated mice, and untreated control mice, and the tissues were analyzed by flow cytometry for detection of inflammatory monocytes. As shown in Figure 19, RS504393 -treated animals had significantly fewer monocytes than untreated animals, thus demonstrating the ability of this drug to suppress monocyte infiltration of lung tissues containing CT26 metastases.

EXAMPLE 14

CCR2 SUPPRESSED GROWTH OF BREAST METASTASES IN MICE

To assess the ability of CCR2 antagonists to suppress the growth of tumor metastases, BALB/c mice with syngeneic breast cancer cell line 4T1 -induced tumors were used as a model system. As shown in Figure 15, tumor metastases were established in BALB/c mice by i.v. injection of 2.5 X 10^5 luciferase-expressing 4T1 cells, and one day after tumor cell injection, once daily treatment with RSI 02895 was initiated at a dose of 5 mg/kg. Figure 20 shows in vivo imaging (IVIS, Perkin Elmer) of the luciferase-expressing tumors at day 9 of the experiment. As quantified in Figure 21, treatment with RSI 02895 resulted in a significant inhibition of tumor growth.

Moreover, in a similar experiment, 4T1 breast cancer metastases were established as described in Figure 15, and beginning twenty-four hours later, RSI 02895 dosing was initiated by providing the mice ad libitum access to water dosed with the drug (5mg/kg/mouse/day based on an estimated consumption of 3 ml/day/mouse). Metastatic tumor growth was monitored by IVIS (as described herein). As shown in Figure 22, a significant suppression of metastases was observed in the mice receiving RSI 02895. Thus,
we have obtained strong evidence of the efficacy of CCR2 antagonists as a novel class of anti-metastatic drugs that can be efficaciously delivered via a number of different routes of administration.

EXAMPLE 15

SYNERGISTIC DRUG COMBINATIONS FOR MONOCYTE MIGRATION INHIBITION

Boyden chamber assays were performed (as described in Mitchell et al. (2012) Int. Immunopharmacol, supra) using the human monocyte cell line THP-1 in response to a CCL2 (MCP-1) gradient to determine whether monocyte migration inhibitors could be combined together to achieve additive or synergistic inhibition of monocyte migration. Synergistic inhibition of monocyte migration activity was very specifically defined in 2-drug combinations, using a statistical definition as assessed using 2-way ANOVA (Prism5 GraphPad software). We compared the monocyte migration inhibition activity of low doses of drugs from each of the 3 classes, using 2-way combinations. Most of the data are presented in typical 2-way ANOVA fashion for assessing drug interactions.

Interactions of Class I drugs (ARBs) with a Class II drug (ondansetron).

Figure 23(a) demonstrates a strong 2-way interaction between ondansetron and irbesartan, as their combination induced a synergistic inhibition of monocyte migration. Figure 23(b) demonstrates a strong 2-way interaction between ondansetron and telmisartan, as their combination induced a synergistic inhibition of monocyte migration. Figure 23(c) demonstrates a weak 2-way interaction between that ondansetron and losartan, as their combination induced a weak synergistic inhibition of monocyte migration. The results of these experiments suggest that Class I and Class II drugs utilize different mechanisms of action for inhibiting monocyte migration. Compositions comprising combinations of drugs from these different classes will likely provide improved in vivo inhibition of monocyte migration, perhaps enabling delivery of decreased doses of the drugs, which may in turn decrease drug-induced toxicity or off-targeting effects.

Interactions of Class I drugs (ARBs) with a Class III drug (RSI 02895).

Figure 24(a) demonstrates a strong 2-way interaction between losartan and RSI 02895, as their combination induced a synergistic inhibition of monocyte migration.
Figure 24(b) demonstrates a weak or no 2-way interaction between telmisartan and RSI 02895, as their combination induced little or no synergistic inhibition of monocyte migration. Figure 24(c) demonstrates a strong 2-way interaction between that irbesartan and RSI 02895, as their combination induced a strong synergistic inhibition of monocyte migration. The results of these experiments suggest that Class I and Class III drugs utilize different mechanisms of action for inhibiting monocyte migration. Compositions comprising combinations of drugs from these different classes will likely provide improved in vivo inhibition of monocyte migration, perhaps enabling delivery of decreased doses of the drugs, which may in turn decrease drug-induced toxicity or off-targeting effects.

**Interactions of a Class II drug (ondansetron) and a Class III drug (RSI02895).**

Figure 25 demonstrates that no interaction between RSI 02895 and ondansetron was observed, thus suggesting the two classes of drugs may have similar mechanisms of action for inhibiting monocyte migration.

**EXAMPLE 16**

**SYNERGISTIC COMBINATION OF LOSARTAN AND SUNITINIB**

To assess the effect of a Losartan / Sunitinib combination therapy approach for tumor treatment, tumors were seeded in mice (BALB/c, n = 5 per group) via intravenous injection with 2.5 X 10^5 K7M2 murine osteosarcoma cells expressing a luciferase reporter gene. Three days later, treatment was started with losartan (6 mg/kg, i.p. BID) and sunitinib (0.8 mg/kg, ip, once daily for 5 days on, 2 off). Drugs were injected separately. Tumor growth was monitored by IVIS imaging every 2-3 days. As shown in Figure 26, tumors in mice receiving the Losartan / Sunitinib combination, had a decreased rate of growth as compared to mice receiving Losartan or Sunitinib alone (data not significant), suggesting that these drugs may interact synergistically to suppress tumor growth and metastasis. At the completion of the experiment, all animals were euthanized and lung tissues collected to evaluate myeloid cell responses by flow cytometry. Figure 27(a) shows the percentage of all monocytes present in the lungs of the mice having lung metastases, and Figure 27(b) shows the percentage of PD-L1+ monocytes present in the lungs of the mice having lung metastases. Using each of these metrics, a trend was observed suggesting that Losartan /
Sunitinib combination results in a synergistic inhibition of monocyte migration to the lung tissue having tumors.

**EXAMPLE 17**

**COMBINATION OF RS102895 AND A CATIONIC LIPID DNA COMPLEX (CLDC)**

To assess the effect of combining a general non-specific immunostimulant with RS102895 for the suppression of primary tumor growth, 1 X 10^6 MCA-205 tumor cells were injected subcutaneously in mice (BALB/c), and one day after tumor challenge, dosing was initiated by intraperitoneal injection of the non-specific immunostimulant cationic lipid dna complex 25 µl administered once every 7 days) alone or in combination with RS102895 (5mg/kg daily). Dosing continued in this manner throughout the experiment. Tumor measurements were made using calipers every 2-3 days. Figure 28 shows that mice receiving CLDC alone or in combination with RS102895 had a decreased rate of primary tumor growth (data not significant).

In a similar experiment designed to test the effect of CLDC in combination with RS102895 for the treatment of metastatic disease, CT26 metastatic tumors were established in mice (as described in Example 11), and the mice were treated with CLDC alone or in combination with RS102895 (as described above in this Example). Tumor growth was followed by IVIS as described in Example 11. Figure 29 shows the IVIS results. Figure 29(a) shows that mice receiving CLDC alone or in combination with RS102895 had a decreased rate of tumor growth (data not significant). Figure 29(b) is the same data as reported in Figure 29(a) zoomed in on the lower datapoints. Analysis in this way shows a nonsignificant trend suggesting a synergistic interaction for tumor growth suppression between CLDC and RS102895. Figure 30 shows survival data for the mice of this experiment, demonstrating a slight survival benefit to the mice receiving RS102895 as compared to the control mice. Taken together, these data suggest that RS102895 can be combined with CLDC to inhibit the growth of primary and metastatic tumors.
EXAMPLE 1 8

GW2580 TREATMENT AT TIME OF VACCINATION SIGNIFICANTLY IMPROVED
HUMORAL AND CELLULAR IMMUNITY

To assess the effects of GW2580 on humoral immunity, mice were treated with GW2580 at the time of vaccination. Briefly, two groups of mice (n=3) were vaccinated with 2 µg of ovalbumin in a CLDC adjuvant. The first group of mice received 160 mg/kg GW2580 via i.p. injection once daily for 2 days immediately after vaccination. The second group of mice received no additional treatment following vaccination. Second vaccinations were repeated for the two groups of mice 10 days following the initial vaccinations, and second GW2580 treatments were repeated for the groups as described for the first cycle of vaccination. Anti-ovalbumin antibodies from each group of mice were titered (as described in Mitchell et al. (2012), J. Immunology, supra) to assess the degree to which humoral immunity was enhanced in the mice receiving GW2580 treatment. As shown in Figure 31, mice treated with GW2580 were found to have significantly higher anti-ovalbumin antibody titers than mice who received no additional treatment with vaccination, indicating that administration of GW2580 in conjunction with the antigen ovalbumin enhanced humoral immunity. The asterisk in Figure 31 indicates indicate statistical differences, i.e., a p-value < 0.05.

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, PCT patent application, PCT patent application publications, foreign patents, foreign patent applications and non-patent publications referred to in this specification or listed in any Application Data Sheet are incorporated herein by reference in their entirety. From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.
REFERENCES CITED


CLAIMS

What is claimed is:

1. A method for enhancing an immune response, decreasing recruitment of monocytes to a lymph node, amplifying vaccine immunity, or inhibiting tumor growth or metastasis, in an individual, the method comprising administering to the individual:

   (a) an angiotensin II receptor blocker (ARB) or a compound of Formula (I):

   \[
   \text{(I)}
   \]

   wherein
   \( R^1 \) is hydrogen or \( \text{C}_{1-6} \) alkyl;
   \( \equiv \) is a single bond or double bond;
   \( Q^1 \) is \( \text{N or CH} \);
   \( R^2 \) is selected from hydrogen and \( \text{C}_{1-6} \) alkyene, wherein one carbon unit of said alkyene is optionally replaced with \( -0-, -S-, -SO-, -SO_{2}-, -\text{NR}^a-, \text{or } -\text{CO}- \); wherein \( R^a \) is hydrogen or \( \text{C}_{1-6} \) alkyl; and
   \( R^3 \) is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \( \text{C}_{1-6} \) alkyl;
   or pharmaceutically acceptable salts thereof; and

   (b) an antigen, a vaccine, or an anti-tumor preparation.

2. The method of claim 1, wherein the method is for enhancing an immune response against an antigen in the individual, and wherein (b) is an antigen.

3. The method of claim 2, wherein the enhanced immune response comprises an enhanced humoral immune response.
4. The method of claim 3, wherein the enhanced humoral immune response comprises an increased antibody titer against the antigen.

5. The method of claim 2, wherein the enhanced immune response comprises an enhanced cellular immune response.

6. The method of claim 5, wherein the enhanced cellular immune response comprises increased release of IFNγ in response to the antigen.

7. The method of claim 2, wherein the enhanced immune response comprises an enhanced humoral immune response and an enhanced cellular immune response.

8. The method of claim 2, wherein the antigen comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

9. The method of claim 2, wherein the ARB or the compound of Formula (I) and the antigen are present in a single pharmaceutical composition.

10. The method of claim 2, wherein the ARB or the compound of Formula (I) is present in a first pharmaceutical composition, and the antigen is present in a second pharmaceutical composition.

11. The method of claim 1, wherein the method is for decreasing recruitment of monocytes to a lymph node in the individual, and wherein (b) is an antigen.

12. The method of claim 11, wherein the monocytes are inflammatory monocytes or CD14^{hi}CD16^{−} human monocytes.

13. The method of claim 11, wherein the lymph node is a draining lymph node.
14. The method of claim 13, wherein the draining lymph node is a vaccine draining lymph node.

15. The method of claim 11, wherein the antigen comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

16. The method of claim 11, wherein the ARB or the compound of Formula (I) and the antigen are present in a single pharmaceutical composition.

17. The method of claim 11, wherein the ARB or the compound of Formula (I) is present in a first pharmaceutical composition and wherein the antigen is present in a second pharmaceutical composition.

18. The method of claim 1, wherein the method is for amplifying vaccine immunity in the individual, and wherein (b) is a vaccine.

19. The method of claim 18, wherein the vaccine comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus-like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

20. The method of claim 18, wherein the ARB or the compound of Formula (I) and the antigen are present in a single pharmaceutical composition.

21. The method of claim 18, wherein the ARB or the compound of Formula (I) is present in a first pharmaceutical composition and wherein the vaccine is present in a second pharmaceutical composition.

22. The method of claim 1, wherein the method is for inhibiting tumor growth or metastasis in the individual, and wherein (b) is an anti-tumor preparation.

23. The method of claim 22, wherein the individual has cancer.
24. The method of claim 23, wherein the cancer is an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.

25. The method of any one of claims 22-24, wherein the anti-tumor preparation comprises a therapeutic antibody, a topoisomerase inhibitor, an antimetabolite, a platinum-based agent, an alkylating agent, a tyrosine kinase inhibitor, an Anthracycline antibiotic, an anti-angiogenic agent, or a vinca alkaloid.

26. The method of claim 22, wherein the ARB or the compound of Formula (I) and the anti-tumor preparation are present in a single pharmaceutical composition.

27. The method of claim 22, wherein the ARB or the compound of Formula (I) is present in a first pharmaceutical composition, and the anti-tumor preparation is present in a second pharmaceutical composition.

28. The method of any one of claims 22-27, further comprising administering to the individual a receptor tyrosine kinase Inhibitor (TKI).

29. The method of claim 28, wherein the TKI is sunitinib.

30. The method of claim 28, wherein administration of the TKI results in an additive increase in the suppression of tumor growth or metastasis.

31. The method of claim 28, wherein administration of the TKI results in a synergistic increase in the suppression of tumor growth or metastasis.

32. The method of any one of claims 9, 16, 20 and 26, wherein the single pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously,
intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

33. The method of any one of claims 10, 17, 21 and 27, wherein the first pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

34. The method of any one of claims 10, 17, 21, 27, and 33 wherein the second pharmaceutical composition is administered orally, via topical application, inhalation, intravenous injection, intra-arterial injection, intramuscular injection, application to a wound site, application to a surgical site, intracavitary injection, by suppository, subcutaneously, intradermally, transcutaneously, by nebulization, intraplurally, intraperitoneally, intraventricularly, intra-articularly, intraocularly, or intraspinally.

35. The method of any one of claims 10, 17, 21, 27, 33, and 34 wherein the first pharmaceutical composition is administered before the second pharmaceutical composition.

36. The method of claim 35, wherein the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another.

37. The method of any one of claims 10, 17, 21, 27, 33, and 34, wherein the first pharmaceutical composition is administered after the second pharmaceutical composition.

38. The method of claim 37, wherein the first and second pharmaceutical compositions are administered within a time period of less than 12 hours of one another.

39. The method of any one of claims 10, 17, 21, 27, 33, and 34, wherein the first pharmaceutical composition and the second pharmaceutical compositions are administered simultaneously.
40. The method of any one of claims 1-39, wherein (a) is the ARB.

41. The method of claim 40, wherein the individual does not have hypertension, congestive heart failure, a history of myocardial infarction, or diabetic nephropathy.

42. The method of claim 40, wherein the individual has not taken the ARB for the treatment of hypertension, congestive heart failure, or diabetic nephropathy.

43. The method of claim 40, wherein the individual does not have a detectable level of the ARB in their blood or urine prior to administration of the ARB in conjunction with the antigen.

44. The method of claim 40, wherein the ARB is Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, Azilsartan, or Valsartan.

45. The method of claim 44, wherein the ARB is Losartan, and wherein the Losartan is administered at a dosage of 30 mg/kg.

46. The method of claim 44, wherein the ARB is Losartan, and wherein the Losartan is administered at a dosage of less than 25 mg.

47. The method of claim 44, wherein the ARB is Candesartan, and wherein the Candesartan is administered at a dosage of less than 4 mg.

48. The method of claim 44, wherein the ARB is Eprosartan, and wherein the Eprosartan is administered at a dosage of less than 400 mg.

49. The method of claim 44, wherein the ARB is Irbesartan, and wherein the Irbesartan is administered at a dosage of less than 150 mg.

50. The method of claim 44, wherein the ARB is Olmesartan, and wherein the Olmesartan is administered at a dosage of less than 20 mg.
51. The method of claim 44, wherein the ARB is Telmisartan, and wherein the Telmisartan is administered at a dosage of less than 20 mg.

52. The method of claim 44, wherein the ARB is Valsartan, and wherein the Valsartan is administered at a dosage of less than 20 mg.

53. The method of claim 44, wherein the ARB is Azilsartan, and wherein the Azilsartan is administered at a dosage of less than 80 mg.

54. The method of any one of claims 1-39, wherein (a) is the compound of Formula (I).

55. The method of claim 54, wherein the compound of Formula (I) is a compound of Formula (II):

![Formula (II)](image)

wherein
R\(^1\) is hydrogen or C\(_{i-6}\) alkyl;
≡≡≡ is a single bond or double bond;
Q\(^1\) is N or CH; and
R\(^4\) is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C\(_{i-6}\) alkyl;
or pharmaceutically acceptable salts thereof.

56. The method of claim 54, wherein the compound of Formula (I) is a compound of Formula (III):

![Formula (III)](image)
wherein

- is a single bond or double bond;

Q¹ is N or CH; and

R⁴ is an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \( \text{C}_{1-6} \) alkyl;

or pharmaceutically acceptable salts thereof.

57. The method of claim 54, wherein the compound of Formula (I) is a compound of Formula (IV):

![Formula IV](image)

wherein

R¹ is hydrogen or \( \text{C}_{1-6} \) alkyl;

- is a single bond or double bond;

Q¹ is N or CH; and

R⁴ is selected from \( \text{N} \), \( \text{N} = \text{N} \), and \( \text{N} \text{H} \);

or pharmaceutically acceptable salts thereof.

58. The method of claim 54, wherein the compound of Formula (I) is a compound of Formula (V):

![Formula V](image)

wherein

- is a single bond or double bond;

Q¹ is N or CH; and
R^4 is selected from and or pharmaceutically acceptable salts thereof.

59. The method of claim 54, wherein the compound of Formula (I) is Ondansetron.

60. The method of claim 59, wherein the Ondansetron is administered at a dosage of 3mg/kg.

61. The method of claim 59, wherein the Ondansetron is administered at a dosage of less than 12 mg.

62. The method of any one of claims 59-61, wherein the individual has not taken the Ondansetron or the Alosetron for the treatment of irritable bowel syndrome (IBS), post-operative nausea and vomiting (PONY), radiation-induced nausea and vomiting (RINV), or chemotherapy-induced nausea and vomiting (CINV).

63. The method of any one of claims 59-61, wherein the individual does not have a detectable level of the Ondansetron in their blood or urine prior to administration of the Ondansetron in conjunction with the antigen.

64. A composition comprising (a) an antigen or a vaccine; and (b) an ARB or a compound of Formula (I):

wherein

R^1 is hydrogen or C_{1-6} alkyl;

=== is a single bond or double bond;
Q¹ is N or CH;
R² is selected from hydrogen and C₁₀ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO₂-, -NR²-, or -CO-; wherein R³ is hydrogen or C₁₀ alkyl; and

5 R³ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C₁₀ alkyl; or pharmaceutically acceptable salts thereof.

65. The composition of claim 64, further comprising a pharmaceutically acceptable carrier.

66. The composition of claim 64 or claim 65, wherein the antigen or the vaccine comprises live whole virus, killed whole virus, attenuated whole virus, killed bacteria, attenuated bacteria, a virus like particle, a bacterial, viral, or parasite protein, a recombinant protein, or a peptide.

67. The composition of any one of claims 64-66, wherein the composition comprises the compound of Formula (I), and, optionally, wherein the compound of Formula (I) is Ondansetron.

68. The composition of any one of claims 64-66, wherein the composition comprises the ARB, and, optionally, wherein the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan.

69. A composition comprising (a) an anti-tumor preparation; and (b) an ARB or a compound of Formula (I):

wherein
R\textsuperscript{1} is hydrogen or C\textsubscript{6}alkyl;

\text{===} is a single bond or double bond;

Q\textsuperscript{1} is N or CH;

R\textsuperscript{2} is selected from hydrogen and C\textsubscript{6}alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO\textsubscript{2}-, -NR\textsubscript{a}-, or -CO-; wherein R\textsuperscript{3} is hydrogen or C\textsubscript{6}alkyl; and

R\textsuperscript{3} is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C\textsubscript{6}alkyl; or pharmaceutically acceptable salts thereof.

70. The composition of claim 69, further comprising a pharmaceutically acceptable carrier.

71. The composition of claim 69 or claim 70, wherein the anti-tumor preparation comprises a therapeutic antibody, a topoisomerase inhibitor, an antimetabolite, a platinum-based agent, an alkylating agent, a tyrosine kinase inhibitor, an Anthracycline antibiotic, an anti-angiogenic agent, or a vinca alkaloid.

72. The composition of any one of claims 69-71, wherein the composition comprises the compound of Formula (I), and, optionally, wherein the compound of Formula (I) is Ondansetron.

73. The composition of any one of claims 69-71, wherein the composition comprises the ARB, and, optionally, the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan.

74. The composition of any one of claims 64-73 further comprising a receptor tyrosine kinase.

75. A kit comprising the composition of any one of claims 64-74 and, optionally, instructions for use.
76. A kit comprising

(a) a vaccine, an antigen, or an anti-tumor preparation; and

(b) a compound of Formula (I):

\[
\begin{align*}
\text{O} & \quad \text{R}^2 - \text{R}^3 \\
\text{R}^1 & \quad \text{N} \quad \text{R}^1
\end{align*}
\]

\( (I) \)

wherein

- \( \text{R}^1 \) is hydrogen or \( \text{C}_{1-6} \) alkyl;
- \( \text{===} \) is a single bond or double bond;
- \( \text{Q}^1 \) is N or CH;
- \( \text{R}^2 \) is selected from hydrogen and \( \text{C}_{1-6} \) alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO\(_2\)-, -NR\(_a\)-, or -CO-; wherein \( \text{R}^a \) is hydrogen or \( \text{C}_{1-6} \) alkyl; and
- \( \text{R}^3 \) is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with \( \text{C}_{1-6} \) alkyl;
- or pharmaceutically acceptable salts thereof; and

(c) optionally, instructions for use,

wherein the compound of Formula (I) is provided in a first container, and wherein the vaccine, antigen, or anti-tumor preparation is provided in a second container.

77. The kit of claim 76, wherein the compound of Formula (I) is Alosetron or Ondansetron.

78. A kit comprising

(a) an ARB;
(b) a vaccine, an antigen, or an anti-tumor preparation; and

c) optionally, instructions for use,

wherein the ARB is provided in a first container, and wherein the vaccine, antigen, or anti-tumor preparation is provided in a second container.

79. The kit of claim 78, wherein the ARB is Azilsartan, Losartan, Candesartan, Eprosartan, Irbesartan, Olmesartan, Telmisartan, or Valsartan.

80. A method of inhibiting monocyte migration in an individual, the method comprising administering to the individual at least two monocyte migration inhibitors (MMIs), wherein at least two of said MMIs have different mechanisms of action for inhibiting monocyte migration.

81. The method of claim 80, wherein the administration of said at least two MMIs results in additive inhibition of monocyte migration.

82. The method of claim 80, wherein the administration of said at least two MMIs results in synergistic inhibition of monocyte migration.

83. The method of any one of claims 80-82, wherein the at least two MMIs are selected from the group consisting of:

(a) MMIs having angiotensin receptor blocking activity (Class I MMIs);

(b) MMIs having serotonin receptor antagonist activity (Class II MMIs); and

(c) MMIs having CCR2 receptor antagonist activity (Class III MMIs)

84. The method of claim 83, wherein the Class I MMI is selected from the group consisting of losartan, irbesartan, and telmisartan.
85. The method of claim 83, wherein the Class II MMI is a compound of Formula (I):

![Chemical Structure](image)

(I)

wherein

- $R^1$ is hydrogen or $C_{i-6}$ alkyl;
- $\equiv$ is a single bond or double bond;
- $Q^1$ is N or CH;
- $R^2$ is selected from hydrogen and $C_{i-6}$ alkyne, wherein one carbon unit of said alkyne is optionally replaced with -O-, -S-, -SO-, -SO$_2$-, -NR$^a$-, or -CO-; wherein $R^a$ is hydrogen or $C_{i-6}$ alkyl; and
- $R^3$ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with $C_{i-6}$ alkyl;

or pharmaceutically acceptable salts thereof.

86. The method of claim 85, wherein the compound of Formula (I) is Ondansetron.

87. The method of claim 83, wherein the Class III MMI is a competitive antagonist of the CCR2 receptor.

88. The method of claim 87, wherein the Class III MMI is RS102895.

89. The method of claim 87, wherein the Class III MMI is RS504393.

90. The method of claim 83, wherein the at least two MMIs comprise a Class I MMI and a Class II MMI.

91. The method of claim 90, wherein the at least two MMIs comprise a combination selected from the group consisting of:
(a) ondansetron and irbesartan;

(b) ondansetron and telmisartan; and

(c) ondansetron and Losartan.

92. The method of claim 83, wherein the at least two MMIs comprise a Class I MMI and a Class III MMI.

93. The method of claim 92, wherein the at least two MMIs comprise a combination selected from the group consisting of:

(a) RSI 02895 and irbesartan;

(b) RSI 02895 and telmisartan; and

(c) RSI 02895 and Losartan.

94. The method of any one of claims 80-93, further comprising administering an antigen or a vaccine to the individual.

95. The method of claim 94, wherein the antigen is administered in conjunction with the at least two MMIs.

96. The method of claim 95, wherein the method enhances an immune response against an antigen in the individual.

97. The method of claim 95, wherein the method decreases recruitment of monocytes to a lymph node in the individual.

98. The method of claim 94, wherein the vaccine is administered in conjunction with the at least two MMIs.
The method of claim 98, wherein the method amplifies vaccine immunity in the individual.

A method of suppressing tumor growth or metastasis in an individual with cancer, the method comprising administering to the individual one or more monocyte migration inhibitors (MMIs).

The method of claim 100, where said one or more MMIs are selected from the group consisting of:

(a) MMIs having angiotensin receptor blocking activity (Class I MMIs);

(b) MMIs having serotonin receptor antagonist activity (Class II MMIs); and

(c) MMIs having CCR2 receptor antagonist activity (Class III MMIs).

The method of claim 101, wherein the Class I MMI is selected from the group consisting of losartan, irbesartan, and telmisartan.

The method of claim 101, wherein the Class II MMI is a compound of Formula (I):

wherein
R¹ is hydrogen or Cᵢ₋₆ alkyl;
═ is a single bond or double bond;
Q¹ is N or CH;
R² is selected from hydrogen and Cᵢ₋₆ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -0-, -S-, -SO-, -SO₂-, -NRᵃ-, or -CO-; wherein Rᵃ is hydrogen or Cᵢ₋₆ alkyl; and
R^3 is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with C_1-6 alkyl;
or pharmaceutically acceptable salts thereof.

104. The method of claim 103, wherein the compound of Formula (I) is Ondansetron.

105. The method of claim 101, wherein the Class III MMI is a competitive antagonist of the CCR2 receptor.

106. The method of claim 105, wherein the Class III MMI is RS102895.

107. The method of claim 105, wherein the Class III MMI is RS504393.

108. The method of any one of claims 100-107, wherein the method further comprises administering to the individual a receptor tyrosine kinase inhibitor (TKI).

109. The method of claim 108, wherein the TKI is sunitinib.

110. The method of claim 108, wherein the TKI is GW2580.

111. The method of any one of claims 108 -110, wherein the administration of the TKI results in an additive increase in the suppression of tumor growth or metastasis.

112. The method of any one of claims 108 -110, wherein the administration of the TKI results in a synergistic increase in the suppression of tumor growth or metastasis.

113. The method of any one of claims 100-112, wherein the cancer is an epithelial cancer, breast cancer, prostate cancer, colon cancer, a hematopoietic cancer, leukemia, lymphoma, a sarcoma, melanoma, a head sarcoma, a neck sarcoma, a squamous cell carcinoma, an osteosarcoma, or a brain tumor.
114. The method of any one of claims 100-112, comprising administering two or more MMIs, where at least two of said MMIs have different mechanisms of action for inhibiting monocyte migration.

115. The method of claim 114, wherein the administration of said two or more MMIs results in a synergistic increase in the suppression of tumor growth or metastasis as compared to the administration of one MMI or two or more MMIs all having the same mechanism of action for inhibiting monocyte migration.

116. A composition comprising a monocyte migration inhibitor (MMIs) and an anti-tumor preparation.

117. A composition comprising two or more monocyte migration inhibitors (MMIs).

118. The composition of claim 117, further comprising an antigen, vaccine, or anti-tumor preparation.

119. The composition of claim 117, wherein at least two of said MMIs have different mechanisms of action for inhibiting monocyte migration.

120. The composition of claim 119, comprising a first MMI and a second MMI, wherein the first MMI is a Class I MMI and the second MMI is a Class II MMI or a Class III MMI.

121. The composition of claim 120, wherein the Class II MMI is a compound of Formula I:

\[
\begin{align*}
\text{III} & = \text{hydrogen or } \text{C}_{1-6} \text{ alkyl;} \\
\ätz & = \text{a single bond or double bond;} \\
\end{align*}
\]

\[
\text{(I)}
\]
Q¹ is N or CH;
R² is selected from hydrogen and Cⁱ⁻⁶ alkylene, wherein one carbon unit of said alkylene is optionally replaced with -O-, -S-, -SO-, -SO²-, -NRᵃ-, or -CO-; wherein Rᵃ is hydrogen or Cⁱ⁻⁶ alkyl; and
R³ is hydrogen or an optionally substituted 5-membered heteroaryl ring; wherein the heteroaryl ring is optionally substituted with Cⁱ⁻⁶ alkyl;
or pharmaceutically acceptable salts thereof.

122. The composition of claim 121, wherein the Class II MMI is Ondansetron.

123. The composition of claim 120, wherein in the Class III MMI is RSI 02895 or RS504393.

124. The composition of any one of claims 120-123, wherein the Class I MMI is selected from the group consisting of irbesartan, telmisartan, and losartan.

125. The composition of any one of claims 116-124, further comprising a receptor tyrosine kinase inhibitor (TKI).

126. The composition of claim 125, wherein the TKI is sunitinib.

127. The composition of claim 125, wherein the TKI is GW2580.

128. The composition of any one of claims 116-124, for use in conjunction with a receptor tyrosine kinase inhibitor.

129. The composition of any one of claims 116-128, further comprising a pharmaceutically acceptable carrier.

130. The composition of claim 116 or 118, further comprising a receptor tyrosine kinase inhibitor and a pharmaceutically acceptable carrier.
131. A kit comprising the composition of any one of claims 116-130, further comprising instructions for use.

132. A kit comprising two different monocyte migration inhibitors (MMIs), wherein the MMIs are provided in separate containers or compartments; and wherein the kit further comprises:

(a) an antigen, a vaccine, or an anti-tumor preparation;

(b) a receptor tyrosine kinase (TKI); or

(c) a TKI and (i) an antigen, (ii) a vaccine, or (iii) an anti-tumor preparation.

133. A kit comprising

(a) a monocyte migration inhibitor (MMI);

(b) an anti-tumor preparation, a receptor tyrosine kinase (TKI), or an anti-tumor preparation and a TKI

(c) optionally, instructions for use,

134. The kit of claim 133, wherein the MMI is provided in a first container, and wherein the anti-tumor preparation, the receptor tyrosine kinase (TKI), or the anti-tumor preparation and the TKI is provided in a second container.

135. The kit of claim 133, wherein the MMI is provided in a first container, the anti-tumor preparation is provided in a second container, and the TKI is provided in a third container.
Figure 1

The diagram shows the number of monocytes/hpf in different conditions: Untreated, CCL2+, CCL2+Los (224 ng/ml), CCL2+Los (112 ng/ml), and CCL2+Los (10 ng/ml). The untreated condition has the lowest monocytes/hpf, followed by CCL2+, and then the treated conditions with varying levels of CCL2+Los.
Figure 2

The figure shows a scatter plot with the average number of cells per 40X field plotted against different treatments. The treatments include Control, MCP-1 alone, and various concentrations of Losartan (10ng/ml, 200ng/ml, 1µg/ml, 10µg/ml). There are error bars indicating the variability in the data. The plot highlights the effect of Losartan on cell counts compared to the control.
Figure 3

![Bar chart showing the number of cells/hpf for different treatments.](image)
Figure 4

*all significantly different

average # cells/40X field

control
30ng/ml MCP-1
20ng/ml RS102895
100ng/ml RS102895
250ng/ml RS102895
500ng/ml RS102895
8μg/ml RS102895

30ng/ml MCP-1
Figure 5
Figure 6

![Diagram showing the comparison of inflammatory monocytes in Unvacc, Vacc, and Vacc + Ond conditions.](chart)
Figure 7
Figure 8a

(Dog 1)
Figure 8b

(Dog 2)

![Graph showing cell counts for different conditions: No CCL2, CCL2+, CCL2/Los-treated. The graph indicates a significant difference among the groups.]
Figure 9

Mean Cells/40X Field

No Tx  CCL2+/preTx  CCL2+/postTx
Figure 10

![Bar chart showing endpoint titers for vaccine alone and Vacc + Losartan. The Vacc + Losartan group has a significantly higher endpoint titer compared to the vaccine alone group.](image-url)
Figure 11

![Bar chart showing endpoint titer (log 10) for Control, Vacc, and Vacc + Ond groups. The Vacc + Ond group has a significantly higher endpoint titer compared to the other groups, indicated by an asterisk (*) on the chart.](image-url)
Figure 12

[Diagram showing IFN-γ levels for vaccine alone and Vacc + Losartan]
Figure 13

![Graph showing tumor area over time for different groups: Control, Vacc, and Vacc + Ond.](image-url)
Figure 14

Vaccine

Vaccine + Ond
Models and dosing

24 hours

i.v. luciferase+ tumor cells

Treatment i.p., s.c., or in water

CT26
4T1
Figure 16

- A-Control
- B-RS102895

* p < 0.01
Figure 17

Control

RS-Treated

DAY 14
Figure 18

- Control
- RS504393

(p = 0.01)
Figure 19

% of all lung cells

Control  RS504393

*
Figure 20
Figure 21

- control
- RS-treated

Photon flux (x 10^5)

Days post tumor cell injection
Figure 22

- Control
- RS-Treated

% with metastases

Days post tumor cell injection
Figure 23

(a) Irbesartan Synergy Data

(b) Telmisartan Synergy Data

(c) Losartan Synergy Data
Figure 24

(a) Losartan Synergy Evaluation

(b) Telmisartan Synergy Evaluation

(c) Irbesartan Synergy Evaluation
Figure 25

![Graph showing cell counts per 40X field for different treatments.](image-url)
Figure 26.

K7M2luc met model

- Control
- Losartan
- Sunitinib
- Combo

Days post tumor cell injection

Photons/s

$1.0 \times 10^6$ to $1.0 \times 10^8$
Figure 27(a)
Figure 27(b)
Figure 28

![Graph showing tumor growth over days post tumor cell injection]

- RS102895
- CLDC
- Combo
- Control

Days post tumor cell injection vs. tumor growth (mm²)
Figure 31

[Bar chart showing Ab titer for control and GW2580 treatments.]