medicines can provide to patients. For example, not completing the recommended course of cancer treatment could lead to a recurrence of the disease and a reduced chance of survival.

Strategies to improve drug compliance include making it more convenient for patients to finish the recommended course of treatment. One of the ways this may be accomplished for patients under immunotherapy treatment is by increasing the duration of time that immunoglobulins are in circulation. The rate of immunoglobulin clearance directly affects the amount and frequency of dosage of the immunoglobulin. Therefore, developing an immunoglobulin that confers increased *in vivo* half-life may decrease the amount and/or frequency of dosage, thus minimizing the inconvenience as well as any additional medical costs.

Accordingly, it would be highly advantageous to have modified immunoglobulins that confer increased *in vivo* half-life for therapeutic purposes. The present invention addresses these and other needs, as will be apparent upon review of the following disclosure.

SUMMARY

The disclosure provides novel IgG variants and uses thereof. A number of IgG variants are provided in the disclosure. For example, the present disclosure discloses novel IgG variants comprising a human IgG Fc region comprising two or more amino acid substitutions relative to a wild-type human IgG Fc region at two or more of amino acid residues 251, 252, 307, 308, 378, 428, 430, 434, and 436, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein at least two of the amino acid substitutions are at amino acid residue 251, 252, 307, 308, 378, 428, 430, 434, or 436, and an amino acid substitution at amino acid residue 251 is a substitution with aspartic acid or glutamic acid, an amino acid substitution at amino acid residue 252 is a substitution with tyrosine, an amino acid substitution at amino acid residue 307 is a substitution with glutamine, an amino acid substitution at amino acid residue 308 is a substitution with proline, an amino acid substitution at amino acid residue 378 is a substitution with valine, an amino acid substitution at amino acid residue 428 is a substitution with leucine, an amino acid substitution at amino acid residue 430 is a substitution with alanine or lysine, an amino acid substitution at amino acid residue 434 is a

substitution with alanine, serine or tyrosine, and an amino acid substitution at amino acid residue 436 is a substitution with isoleucine. In a first aspect, there is provided a nucleic acid encoding a variant IgG comprising a human IgG Fc region comprising two or more amino acid substitutions relative to a wild-type human IgG Fc region at two or more of amino acid residues 251, 252, 307, 308, 378, 380, 428, 430, 434, and 436, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein the Fc region comprises:

an amino acid substitution at amino acid 251 with aspartic acid and an amino acid substitution at 434 with histidine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 378 with valine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 436 with isoleucine;

an amino acid substitution at amino acid 308 with proline and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 308 with proline and an amino acid substitution at 434 with tyrosine;

an amino acid substitution at amino acid 378 with valine and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 434 with alanine and an amino acid substitution at 436 with isoleucine;

an amino acid substitution at amino acid 251 with aspartic acid, an amino acid substitution at amino acid 307 with glutamine, and an amino acid substitution at 434 with histidine;

an amino acid substitution at amino acid 252 with tyrosine, an amino acid substitution at amino acid 308 with proline, and an amino acid substitution at 434 with tyrosine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 380 with alanine, and an amino acid substitution at 434 with serine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 380 with alanine, and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 378 with valine, and an amino acid substitution at 436 with isoleucine; or

an amino acid substitution at amino acid 251 with aspartic acid, an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 428 with leucine, an amino acid substitution at amino acid 434 with histidine, and an amino acid substitution at 436 with isoleucine.

In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 251, wherein the amino acid substitution at amino acid 251 is the substitution with aspartic acid or glutamic acid. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 307, wherein the amino acid substitution at amino acid 307 is the substitution with glutamine. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 308, wherein the amino acid substitution at amino acid 308 is the substitution with proline. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 378, wherein the amino acid substitution at amino acid 378 is the substitution with valine. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 436, wherein the amino acid substitution at amino acid 436 is the substitution with isoleucine. In certain embodiments, the variant IgGs have a higher binding affinity for FcRn than the IgG having the wild-type human IgG Fc region.

In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 307 with glutamine and the amino acid substitution at amino acid 434 with alanine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 307 with glutamine and the amino acid substitution at amino acid 434 with serine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 308 with proline and the amino acid substitution at amino acid

434 with alanine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 252 with tyrosine and the amino acid substitution at amino acid 434 with alanine. In one embodiment, human IgG Fc region comprises the amino acid substitution at amino acid 378 with valine and the amino acid substitution at amino acid 434 with alanine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 428 with leucine and the amino acid substitution at amino acid 434 with alanine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 434 with alanine and the amino acid substitution at amino acid 436 with isoleucine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 308 with proline and the amino acid substitution at amino acid 434 with tyrosine. In one embodiment, the human IgG Fc region comprises the amino

acid substitution at amino acid 307 with glutamine and the amino acid substitution at amino acid 436 with isoleucine.

The present disclosure also discloses novel IgG variants comprising a human IgG Fc region comprising three or more amino acid substitutions relative to a wild-type human IgG Fc region at three or more of amino acid residues 251, 252, 307, 308, 378, 380, 428, 430, 434, and 436, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein at least three of the amino acid substitutions are at amino acid residue 251, 252, 307, 308, 378, 380, 428, 430, 434, or 436, and an amino acid substitution at amino acid residue 251 is a substitution with aspartic acid or glutamic acid, an amino acid substitution at amino acid residue 252 is a substitution with tyrosine, an amino acid substitution at amino acid residue 307 is a substitution with glutamine, an amino acid substitution at amino acid residue 308 is a substitution with proline, an amino acid substitution at amino acid residue 378 is a substitution with valine, an amino acid substitution at amino acid residue 380 is a substitution with alanine, an amino acid substitution at amino acid residue 428 is a substitution with leucine, an amino acid substitution at amino acid residue 430 is a substitution with alanine or lysine, an amino acid substitution at amino acid residue 434 is a substitution with alanine, serine, tyrosine or histidine, and an amino acid substitution at amino acid residue 436 is a substitution with isoleucine.

In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 251, wherein the amino acid substitution at amino acid 251 is the substitution with aspartic acid or glutamic acid. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 307, wherein the amino acid substitution at amino acid 307 is the substitution with glutamine. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 308, wherein the amino acid substitution at amino acid 308 is the substitution with proline. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 378, wherein the amino acid substitution at amino acid 378 is the substitution with valine. In certain embodiments, the human IgG Fc region comprises an amino acid substitution at amino acid 436, wherein the amino acid substitution at amino acid 436 is the substitution with isoleucine. In certain embodiments, the variant IgGs have a higher binding affinity to FcRn compared to the IgG having the wild-type human IgG Fc region.

In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 307 with glutamine, the amino acid substitution at amino acid 380 with alanine and the amino acid substitution at amino acid 434 with serine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 307 with glutamine, the amino acid substitution at amino acid 380 with alanine and the amino acid substitution at amino acid 434 with alanine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 252 with tyrosine, the amino acid substitution at amino acid 308 with proline and the amino acid substitution at amino acid 434 with tyrosine. In one embodiment, the human IgG Fc region comprises the amino acid substitution at amino acid 251 with aspartic acid, the amino acid substitution at amino acid 307 with glutamine and the amino acid substitution at amino acid 434 with histidine.

In certain embodiments, the present disclosure provides the variant IgGs or fragments thereof further comprising an amino acid substitution at position 297 to alanine.

In certain embodiments, the variant IgG of the present disclosure has a higher binding affinity for FcRn than the IgG having the wild-type human IgG Fc region. In certain embodiments, the variant IgG has a higher binding affinity for FcRn at pH 6.0 than at pH 7.4. In certain embodiments, the variant IgG is a human or humanized IgG. In certain embodiments, the variant IgG is IgG_1, IgG_2, IgG_3 or IgG_4. In certain embodiments, the IgG Fc region of the variant IgG is an IgG_1, IgG_2, IgG_3 or IgG_4 Fc region. In certain embodiments, the IgG Fc region of the variant IgG is an IgG_1 Fc region.

In certain embodiments, the variant IgG is an anti-VEGF antibody. In certain embodiments, the variant IgG is a variant of bevacizumab. In certain embodiments, the IgG having the wild-type human IgG Fc region is bevacizumab. In certain embodiments, the wild-type human IgG Fc region is the Fc region of bevacizumab. In certain embodiments, the variant IgG comprises the heavy chain variable domain (SEQ ID NO:1) and light chain variable domain (SEQ ID NO:2). In certain embodiments, the variant IgG comprises the heavy chain variable domain (SEQ ID NO:3) and light chain variable domain (SEQ ID NO:4). In certain embodiments, the variant IgG comprises the heavy chain variable domain (SEQ ID NO:7) and light chain variable domain (SEQ ID NO:8).

The present disclosure further discloses pharmaceutical compositions comprising any of the variant IgGs described herein and a pharmaceutically acceptable carrier. A kit comprising any of the variant IgGs described herein, in a container, and instructions for use is also provided herein.

acid substitution at amino acid residue 307 is a substitution with glutamine, and the amino acid substitution at amino acid residue 434 is a substitution with serine.

In another embodiment, variant IgGs comprising a human IgG Fc region are provided, wherein the human IgG Fc region comprises amino acid substitutions relative to a wild-type human IgG Fc region at amino acid residues 308 and 434, numbered according to the EU index as in Kabat, wherein the variant IgG_1 has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein the amino acid substitution at amino acid residue 308 is a substitution with proline, and the amino acid substitution at amino acid residue 434 is a substitution with alanine. In a second aspect, there is provided a nucleic acid encoding a human IgG1 Fc region comprising amino acid substitutions relative to a wild-type human IgG1 Fc region at amino acid residues 308 and 434, numbered according to the EU index as in Kabat, wherein the variant IgG1 has an increased half-life compared to the half-life of an IgG1 having the wild-type human IgG1 Fc region, and wherein the amino acid substitution at amino acid residue 308 is a substitution with proline, and the amino acid substitution at amino acid residue 434 is a substitution with alanine.

In another embodiment, variant IgGs comprising a human IgG Fc region are provided, wherein the human IgG Fc region comprises amino acid substitutions relative to a wild-type human IgG Fc region at amino acid residues 307, 380 and 434, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein the amino acid substitution at amino acid residue 307 is a substitution with glutamine, the amino acid substitution at amino acid residue 380 is a substitution with alanine, and the amino acid substitution at amino acid residue 434 is a substitution with serine.

In certain embodiments, the variant IgG comprising a human IgG Fc region described above has a higher binding affinity for FcRn than the IgG having the wild-type human IgG Fc region. In certain embodiments, the variant IgG has a higher binding affinity for FcRn at pH 6.0 than at pH 7.4. In certain embodiments, the variant IgG is a human or humanized IgG. In certain embodiments, the variant IgG is IgG_1, IgG_2, IgG_3 or IgG_4. In certain embodiments, the IgG Fc region of the variant IgG is an IgG_1, IgG_2, IgG_3 or IgG_4 Fc region. In certain embodiments, the IgG Fc region of the variant IgG is IgG_1 Fc region. In certain embodiments, the variant IgG is an anti-VEGF antibody. In certain embodiments, the variant IgG is a variant of bevacizumab. In certain embodiments, the IgG having the wild-type human IgG Fc region is bevacizumab. In certain embodiments, the wild-type human IgG Fc region is the Fc region of bevacizumab. In certain embodiments, the variant IgG comprises the heavy chain variable domain (SEQ ID NO:1) and light chain variable domain (SEQ ID NO:2). In certain embodiments, a pharmaceutical composition comprising any of the variant IgGs comprising a human IgG Fc region and a pharmaceutically acceptable carrier are provided herein. A kit comprising any of the

variant IgGs comprising a human IgG Fc region, in a container, and instructions for use is also provided herein.

In certain embodiments, variant IgGs comprising a human IgG_1 Fc region are provided, wherein the variant IgGs comprise the heavy chain variable domain (SEQ ID NO:1) and light chain variable domain (SEQ ID NO:2) and wherein the human IgG_1 Fc region comprises an amino acid substitution relative to a wild-type human IgG_1 Fc region at amino acid residue 434, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG_1 Fc region, and the variant IgG has a higher binding affinity for FcRn compared to binding affinity for FcRn of the IgG having the wild-type human IgG_1 Fc region, and wherein the amino acid substitution at amino acid residue 434 is a substitution with histidine. In certain embodiments, the variant IgG is variant IgG_1.

In certain embodiments, the half life of a variant IgG of the present disclosure is increased by at least 50, 55. 60, 65, 70, 75, 80, 85, 90, 95, or 100% compared to the half life of the IgG having the wild-type human IgG Fc region. In one embodiment, the half life of a variant IgG of the present disclosure is increased by at least 50% compared to the half life of the IgG having the wild-type human IgG Fc region. In another embodiment, the half life of a variant IgG of the present disclosure is increased by at least 75% compared to the half life of the IgG having the wild-type human IgG Fc region. In yet another embodiment, the half life of a variant IgG of the present disclosure is increased by at least 100% compared to the half life of the IgG having the wild-type human IgG Fc region.

In certain embodiments, the half life of a variant IgG of the present disclosure is at least about 15, 20, 25, 30, 35, or 40 days. In one embodiment, the half life of a variant IgG of the present disclosure is at least about 15 days. In another embodiment, the half life of a variant IgG of the present disclosure is at least about 20 days. In another embodiment, the half life of a variant IgG of the present disclosure is at least about 25 days. In another embodiment, the half life of a variant IgG of the present disclosure is at least about 30 days. In another embodiment, the half life of a variant IgG of the present disclosure is at least about 35 days. In another embodiment, the half life of a variant IgG of the present disclosure is at least about 40 days. In certain embodiments, the variant IgG is variant IgG_1.

In certain embodiments, the half life of a variant IgG of the present disclosure is the half life as measured in humans. In certain embodiments, the half life of a variant IgG of the present disclosure is the half life as measured in cynomolgus monkeys. In certain

embodiments, the wild-type IgG or the IgG having the wild-type human IgG Fc region is bevacizumab. In certain embodiments, the half-life of bevacizumab is about 10 to 12 days as measured in cynomolgus monkeys, or about 20 days as measured in humans.

In a third aspect, there is provided an expression vector comprising any of the nucleic acid disclosed herein.

In a fourth aspect, there is provided a host cell comprising the expression vector of the third aspect.

In a fifth aspect, there is provided a method of producing a variant IgG comprising culturing the host cell of the third aspect under conditions where the variant IgG is expressed.

In a sixth aspect, there is provided a variant IgG produced by the method of the fifth aspect.

A number of methods of using IgG variants are provided in the disclosure. Methods of treating tumor in a subject are provided. For example, methods comprise administering to the subject an effective amount of any variant IgGs described above and herein. In certain embodiments, the variant IgG is an anti-VEGF antibody. In certain embodiments, the variant IgG is a variant of bevacizumab. In certain embodiments, the methods further comprise administering to the subject an effective amount of a chemotherapeutic agent.

Methods of inhibiting VEGF activity in a subject are provided in the disclosure. For example, methods comprise administering to said subject an effective amount of any variant IgGs described above and herein. In certain embodiments, the VEGF activity is angiogenesis.

Methods of modulating vascular permeability in a subject are provided in the disclosure. For example, methods comprise administering to said subject an effective amount of any variant IgGs described above and herein.

Methods of treating a non-neoplastic disorder in a subject are provided in the disclosure. For example, methods comprise administering to said subject an effective amount of any variant IgGs described above and herein. In certain embodiments, the non-neoplastic disorder is an autoimmune disease. In certain embodiments, the non-neoplastic disorder is Alzheimer's disease. In certain embodiments, the subject is diagnosed with age-related macular degeneration.

Methods of treating a HER expressing tumor in a subject are provided in the disclosure. For example, methods comprise administering to said subject an effective amount of any variant IgGs described above and herein. In certain embodiments, the variant IgG comprises the heavy chain variable domain (SEQ ID NO:7) and light chain variable domain (SEQ ID NO:8).

Methods of inhibiting or preventing growth of cancer cells in a subject are provided in the disclosure. For example, methods comprise administering to said subject an effective amount of any variant IgGs described above and herein.

Methods of administering to a subject an effective amount of a variant IgG are provided in the disclosure. These methods of administration can be used in combination with other methods (*e.g.*, methods of treatments) described herein. In certain embodiments,

In certain embodiments, the variant IgGs of the disclosure are administered to the subject intravenously. In certain embodiments, the variant IgGs of the disclosure are administered to the subject subcutaneously.

In certain embodiments of the methods described herein, the subject is human. In certain embodiments, the subject is diagnosed with cancer. In certain embodiments, the cancer is selected from the group consisting of non-small cell lung cancer, renal cell carcinoma, ovarian cancer, glioblastoma, breast cancer, and colorectal cancer.

Also provided herein are methods of treating a benign, pre-cancerous or non-metastatic cancer in a subject, which comprise administering to the subject an effective amount of a variant IgG. In certain embodiments, the administration of the variant IgG prevents the benign, pre-cancerous, or non-metastatic cancer from becoming an invasive or metastatic cancer. For example, the benign, pre-cancerous or non-metastatic cancer can be a stage 0, stage I, or stage II cancer, and in certain embodiments, the administration of the variant IgG prevents the benign, pre-cancerous or non-metastatic cancer from progressing to the next stage(s), *e.g.*, a stage I, a stage II, a stage III or stage IV cancer. In certain embodiments, the variant IgG is administered for a time and in an amount sufficient to treat the benign, pre-cancerous, or non-metastatic tumor in the subject or to prevent the benign, pre-cancerous, or non-metastatic tumor from becoming an invasive or metastatic cancer. In certain embodiments, administering the variant IgG reduces tumor size, tumor burden, or the tumor number of the benign, pre-cancerous, or non-metastatic tumor. The variant IgG can also be administered in an amount and for a time to decrease the vascular density in the benign, pre-cancerous, or non-metastatic tumor.

As described herein, the methods of the disclosure can be used to treat, *e.g.,* a stage 0 (*e.g.*, a carcinoma in situ), stage I, or stage II cancer. The methods of neoadjuvant and adjuvant therapy can be used to treat any type of cancer, *e.g.*, benign or malignant. In certain embodiments of the disclosure, the cancer is a solid tumor, including, but not limited to, colon cancer, breast cancer, prostate cancer, renal cancer, lung cancer (*e.g.*, non-small cell lung cancer), melanoma, ovarian cancer, pancreatic cancer, gastrointestinal cancer, head and neck cancer, liver cancer and soft tissue cancers (*e.g.*, B cell lymphomas such as NHL and multiple myeloma and leukemias such as chronic lymphocytic leukemia). In another embodiment, the benign, pre-cancerous, or non-metastatic tumor is a polyp, adenoma, fibroma, lipoma, gastrinoma, insulinoma, chondroma, osteoma, hemangioma, lymphangioma, meningioma, leiomyoma, rhabdomyoma, squamous cell papilloma,

acoustic neuromas, neurofibroma, bile duct cystanoma, leiomyomas, mesotheliomas, teratomas, myxomas, trachomas, granulomas, hamartoma, transitional cell papilloma, pleiomorphic adenoma of the salivary gland, desmoid tumor, dermoid cystpapilloma, cystadenoma, focal nodular hyperplasia, or a nodular regenerative hyperplasia. In another embodiment, the method is desirably used to treat an adenoma. Non-limiting examples of adenomas include liver cell adenoma, renal adenoma, metanephric adenoma, bronchial adenoma, alveolar adenoma, adrenal adenoma, pituitary adenoma, parathyroid adenoma, pancreatic adenoma, salivary gland adenoma, hepatocellular adenoma, gastrointestinal adenoma, tubular adenoma, and bile duct adenoma.

The disclosure also features methods that comprise administering to a subject an effective amount of a variant IgG to prevent occurrence or recurrence of a benign, pre-cancerous, or non-metastatic cancer in the subject. In certain embodiments of the disclosure, the subject is at risk for cancer, polyps, or a cancer syndrome. In one example, the subject has a family history of cancer, polyps, or an inherited cancer syndrome. In certain embodiments of the disclosure, the subject is at risk of developing a benign, pre-cancerous, or non-metastatic tumor. In certain embodiments, the method prevents occurrence or recurrence of said benign, pre-cancerous or non-metastatic cancer in a subject who has never had a tumor, a subject who has never had a clinically detectable cancer, or a subject who has only had a benign tumor.

In another embodiment, methods of preventing or reducing the likelihood of recurrence of a cancer in a subject that includes administering to the subject a variant IgG for a time and in an amount sufficient to prevent or reduce the likelihood of cancer recurrence in the subject are provided. The disclosure includes a method of preventing the recurrence of a cancer in a subject having a tumor that includes the steps of removing the tumor (*e.g.*, using definitive surgery) and thereafter administering to the subject a variant IgG. The disclosure includes methods of preventing the regrowth of a tumor in a subject that includes the steps of removing the tumor (*e.g.*, using definitive surgery) and thereafter administering to the subject a variant IgG. In a related embodiment, the disclosure includes a method of preventing recurrence of cancer in a subject or reducing the likelihood of cancer recurrence in a subject that optionally includes administering to the subject an effective amount of a variant IgG prior to surgery, performing definitive surgery, and administering an effective amount of a variant IgG following the surgery wherein the administration of the variant IgG after the surgery prevents recurrence of the cancer or reduces the likelihood of cancer recurrence. In

another related embodiment, the disclosure includes a method of preventing recurrence of cancer in a subject or reducing the likelihood of cancer recurrence in a subject that includes administering to the subject an effective amount of a variant IgG in the absence of any additional anti-cancer therapeutic agent, wherein the administering prevents recurrence of cancer in a subject or reduces the likelihood of cancer recurrence in a subject.

For each of the above embodiments, the tumor can be any type of tumor including but not limited to the solid tumors, and particularly the tumors and adenomas, described herein. The subject can have a dormant tumor or micrometastases, which may or may not be clinically detectable. In one embodiment of this embodiment, the variant IgG is administered for a time and in an amount sufficient to reduce neovascularization of a dormant tumor or micrometastases. In another embodiment, the variant IgG is administered for a time and in an amount sufficient to prevent occurrence of a clinically detectable tumor, or metastasis thereof, or to increase the duration of survival of the subject.

In one embodiment, the variant IgG is a monotherapy. In another embodiment, the subject has been previously treated for the tumor, for example, using an anti-cancer therapy. In one example, the anti-cancer therapy is surgery. In another embodiment, the subject can be further treated with an additional anti-cancer therapy before, during (*e.g.*, simultaneously), or after administration of the variant IgG. Examples of anti-cancer therapies include, without limitation, surgery, radiation therapy (radiotherapy), biotherapy, immunotherapy, chemotherapy, or a combination of these therapies.

In embodiments where the subject has undergone definitive surgery, the variant IgG is generally administered after a period of time in which the subject has recovered from the surgery. This period of time can include the period required for wound healing or healing of the surgical incision, the time period required to reduce the risk of wound dehiscence, or the time period required for the subject to return to a level of health essentially similar to or better than the level of health prior to the surgery. The period between the completion of the definitive surgery and the first administration of the variant IgG can also include the period needed for a drug holiday, wherein the subject requires or requests a period of time between therapeutic regimes. Generally, the time period between completion of definitive surgery and the commencement of the variant IgG therapy can include less than one week, 1 week, 2 weeks, 3 weeks, 4 weeks (28 days), 5 weeks, 6 weeks, 7 weeks, 8 weeks, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, or more. In one embodiment, the period of time between definitive

surgery and administering the variant IgG is greater than 2 weeks and less than 1 year. In one embodiment, the period of time between definitive surgery and administering the variant IgG is greater than 4 weeks (28 days).

In certain embodiments, each of the above embodiments can further include monitoring the subject for recurrence of the cancer.

The disclosure also provides methods of neoadjuvant therapy prior to the surgical removal of operable cancer in a subject, *e.g.*, a human patient, comprising administering to the patient an effective amount of a variant IgG where the patient has been diagnosed with a tumor or cancer. The variant IgG can be administered alone or in combination with at least one chemotherapeutic agent.

The disclosure also includes a method of treating a subject with operable cancer that includes administering to the subject an effective amount of a variant IgG prior to surgery and thereafter performing surgery whereby the cancer is resected. In one embodiment, the method further includes the step of administering to the subject an effective amount of a variant IgG after surgery to prevent recurrence of the cancer.

In another embodiment, the disclosure concerns a method of neoadjuvant therapy comprising administering to a subject with operable cancer an effective amount of a variant IgG and at least one chemotherapeutic agent prior to definitive surgery.

In another embodiment, the disclosure includes a method of reducing tumor size in a subject having an unresectable tumor comprising administering to the subject an effective amount of a variant IgG wherein the administering reduces the tumor size thereby allowing complete resection of the tumor. In one embodiment, the method further includes administering to the subject an effective amount of a variant IgG after complete resection of the tumor.

In another embodiment, the disclosure concerns a method of treating cancer in a subject comprising the following steps: a) a first stage comprising a plurality of treatment cycles wherein each cycle comprises administering to the subject an effective amount of a variant IgG and at least one chemotherapeutic agent at a predetermined interval; b) a definitive surgery whereby the cancer is removed; and c) a second stage comprising a plurality of maintenance cycles wherein each cycle comprises administering to the subject an effective amount of a variant IgG without any chemotherapeutic agent at a predetermined interval. In one embodiment, the first stage comprises a first plurality of treatment cycles wherein a variant IgG and a first chemotherapy regimen are administered followed by a second

plurality of treatment cycles wherein a variant IgG and a second chemotherapy regimen are administered.

The disclosure provides methods comprising administering to a subject with metastatic or nonmetastatic cancer, following definitive surgery, an effective amount of a variant IgG. In certain embodiments, the method further includes the use of at least one chemotherapeutic agent.

In one embodiment, the method comprises the following steps: a) a first stage comprising a plurality of treatment cycles wherein each cycle comprises administering to the subject an effective amount of a variant IgG and at least one chemotherapeutic agent at a predetermined interval; and b) a second stage comprising a plurality of maintenance cycles wherein each cycle comprises administering to the subject an effective amount of a variant IgG without any chemotherapeutic agent at a predetermined interval. In one embodiment, the first stage comprises a first plurality of treatment cycles wherein a variant IgG and a first chemotherapy regimen are administered, followed by a second plurality of treatment cycles wherein a variant IgG and a second chemotherapy regimen are administered.

In certain embodiments, the variant IgG is an anti-VEGF antibody that binds to VEGF or reduces VEGF expression or biological activity. The anti-VEGF antibody, or antigen-binding fragment thereof, can be a monoclonal antibody, a chimeric antibody, a fully human antibody, or a humanized antibody. In certain embodiments, exemplary antibodies useful in the methods of the disclosure include bevacizumab (AVASTIN®), G6-31, B20-4.1, B20-4.1.1, and fragments thereof.

In certain embodiments, the variant IgG is humanized anti-HER2 monoclonal antibody HERCEPTIN®. In certain embodiments, the variant IgG is chimeric anti-CD20 antibody Rituxan®, anti-IgE antibody XOLAIR®, anti-CD20 antibody, anti-CD11a antibody Raptiva®, anti-Her2 antibody Omnitarg®, an anti-oxLDL antibody, anti-CD4 antibody MTRX1011A, an anti-HCV antibody, an anti- IL-17A/F antibody, an anti-A-beta antibody, an anti-DR6 antibody, anti-human cytomegalovirus (HCMV) antibody, anti-HER receptor family antibody, an anti-tissue factor antibody, MLN-02 antibody, humanized anti-CD 18 $F(ab')_2$ antibody, or a humanized anti-IgE IgG_1 antibody rhuMab-E25. In certain embodiments, the variant IgG is a bispecific antibody wherein target antigens are IL-4 and IL-13. In certain embodiments, the variant IgG is an antibody targeting an epitope of staph aureus.

Although the subject can be treated in a number of different ways prior to, during, or after the administration of the variant IgG, in certain embodiments, the subject is treated without surgery or chemotherapy. In other embodiments, treatment with the variant IgG is a monotherapy or a monotherapy for the duration of the variant IgG treatment period, as assessed by the clinician or described herein.

In other embodiments, treatment with the variant IgG is in combination with an additional anti-cancer therapy, including but not limited to, surgery, radiation therapy, chemotherapy, differentiating therapy, biotherapy, immune therapy, an angiogenesis inhibitor, and an anti-proliferative compound. Treatment with the variant IgG can also include any combination of the above types of therapeutic regimens. In certain embodiments, cytotoxic agents, anti-angiogenic and anti-proliferative agents can be used in combination with the variant IgG. In one embodiment, the anti-cancer therapy is chemotherapy. In certain embodiments, the chemotherapeutic agent and the variant IgG are administered concurrently.

In certain embodiments, the methods of the disclosure are advantageous in treating and preventing early stage tumors, thereby preventing progression to the more advanced stages resulting in a reduction in the morbidity and mortality associated with advanced cancer. The method of the disclosure are also advantageous in preventing the recurrence of a tumor or the regrowth of a tumor, for example, a dormant tumor that persists after removal of the primary tumor, or in reducing or preventing the occurrence or proliferation of micrometastases.

For the methods of the disclosure, the cancer may be a solid tumor, *e.g.*, such as, breast cancer, colorectal cancer, rectal cancer, lung cancer, renal cell cancer, a glioma (*e.g.*, anaplastic astrocytoma, anaplastic oligoastrocytoma, anaplastic oligodendroglioma, glioblastoma multiforme), kidney cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, carcinoid carcinoma, head and neck cancer, melanoma, and ovarian cancer.

The methods of the disclosure can also include monitoring the subject for recurrence of the cancer or tumor.

Other features and advantages of the disclosure will be apparent from the following Detailed Description, the drawings, and the claims.

Any embodiment described herein or any combination thereof applies to any and all variant IgGs and methods of the disclosure described herein.

WHAT IS CLAIMED IS:

1. A nucleic acid encoding a variant IgG comprising a human IgG1 Fc region comprising two or more amino acid substitutions relative to a wild-type human IgG1 Fc region at two or more of amino acid residues 251, 252, 307, 308, 378, 380, 428, 430, 434, and 436, numbered according to the EU index as in Kabat, wherein the variant IgG has an increased half-life compared to the half-life of an IgG having the wild-type human IgG Fc region, and wherein the Fc region comprises:

an amino acid substitution at amino acid 251 with aspartic acid and an amino acid substitution at 434 with histidine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 378 with valine;

an amino acid substitution at amino acid 307 with glutamine and an amino acid substitution at 436 with isoleucine;

an amino acid substitution at amino acid 308 with proline and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 308 with proline and an amino acid substitution at 434 with tyrosine;

an amino acid substitution at amino acid 378 with valine and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 434 with alanine and an amino acid substitution at 436 with isoleucine;

an amino acid substitution at amino acid 251 with aspartic acid, an amino acid substitution at amino acid 307 with glutamine, and an amino acid substitution at 434 with histidine;

an amino acid substitution at amino acid 252 with tyrosine, an amino acid substitution at amino acid 308 with proline, and an amino acid substitution at 434 with tyrosine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 380 with alanine, and an amino acid substitution at 434 with serine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 380 with alanine, and an amino acid substitution at 434 with alanine;

an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 378 with valine, and an amino acid substitution at 436 with isoleucine; or

an amino acid substitution at amino acid 251 with aspartic acid, an amino acid substitution at amino acid 307 with glutamine, an amino acid substitution at amino acid 428 with leucine, an amino acid substitution at amino acid 434 with histidine, and an amino acid substitution at 436 with isoleucine.

2. The nucleic acid of claim 1, wherein the variant IgG comprises the amino acid substitution at amino acid 308 with proline and the amino acid substitution at amino acid 434 with alanine.

3. The nucleic acid of claim 1 or claim 2, wherein the variant IgG has a higher binding affinity for FcRn than the IgG having the wild-type human IgG1 Fc region.

4. The nucleic acid of any one of claims 1 to 3, wherein the variant IgG has a higher binding affinity for FcRn at pH 6.0 than at pH 7.4.

5. The nucleic acid of claims 1 to 4, wherein the variant IgG has an equal or higher efficacy than the IgG having the wild-type human IgG1 Fc region.

6. The nucleic acid of claim 5, wherein the variant IgG has a higher efficacy than the IgG having the wild-type human IgG1 Fc region.

7. The nucleic acid of any one of claims 1 to 6, wherein the variant IgG is a human or humanized IgG.

8. The nucleic acid of any one of claims 1 to 7, wherein the variant IgG is an anti-VEGF antibody.

9. The nucleic acid of any one of claims 1 to 8, wherein the variant IgG is a variant of bevacizumab.

10. The nucleic acid of any one of claims 1 to 9, wherein the variant IgG comprises the heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:1 and the light chain variable domain comprising the amino acid sequence of SEQ ID NO:2.

11. A nucleic acid encoding a human IgG1 Fc region comprising amino acid substitutions relative to a wild-type human IgG_1 Fc region at amino acid residues 308 and 434, numbered according to the EU index as in Kabat, wherein the variant IgG_1 has an increased half-life compared to the half-life of an IgG_1 having the wild-type human IgG_1 Fc region, and wherein the amino acid substitution at amino acid residue 308 is a substitution with proline, and the amino acid substitution at amino acid residue 434 is a substitution with alanine.

12. An expression vector comprising the nucleic acid of any one of claims 1 to 11.

13. A host cell comprising the expression vector of claim 12.

14. A method of producing a variant IgG comprising culturing the host cell of claim 13 under conditions where the variant IgG is expressed.

15. The method of claim 14, further comprising isolating the expressed variant IgG.

16. A variant IgG produced by the method of claim 14 or claim 15.