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(54) Title: METHODS OF TREATING LUNG CANCER WITH A PD-1 AXIS BINDING ANTAGONIST, AN ANTIMETABOLITE, AND A PLATINUM AGENT

(57) Abstract: The present disclosure provides methods for treating lung cancer (such as non-small cell lung cancer, e.g., Stage IV non-squamous non-small cell lung cancer) in an individual. The methods comprise administering to the individual a PD-1 axis binding antagonist (such as an anti-PD-L1 antibody, e.g., atezolizumab), an antimetabolite (e.g., pemetrexed), and a platinum agent (e.g., cisplatin or carboplatin).



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METHODS OF TREATING LUNG CANCER WITH A PD-1 AXIS BINDING ANTAGONIST, AN ANTIMETABOLITE, AND A PLATINUM AGENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/700,184, filed July 18, 2018, and 62/734,936, filed on September 21, 2018; the contents of each of which are hereby incorporated by reference in their entirety.

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 146392045140SEQLIST.TXT, date recorded: July 12, 2019, size: 37 KB).

FIELD

[0003] The present disclosure relates to methods of treating cancers by administering a PD-1 axis binding antagonist (*e.g.*, atezolizumab) in combination with an antimetabolite (*e.g.*, pemetrexed) and a platinum agent (*e.g.*, carboplatin or cisplatin).

BACKGROUND

[0004] Lung cancer remains the leading cause of cancer deaths worldwide; it is the most common cancer in men and accounted for approximately 13% of all new cancers in 2008 (Jemal *et al.* (2011) *CA Cancer J. Clin* 61: 69-90). In 2012, it was estimated that there were 313,000 new cases of lung cancer and 268,000 lung cancer deaths in Europe (GLOBOCAN (2012). Estimated cancer incidence: mortality and prevalence Worldwide in 2012. Available at: [globocan\(dot\)iarc\(dot\)fr/Pages/fact_sheets_cancer.aspx](http://globocan(dot)iarc(dot)fr/Pages/fact_sheets_cancer.aspx)). Similar data from the United States estimated that there would be 221,200 new cases of lung cancer and 158,040 lung cancer deaths in 2015 (Siegel *et al.* (2015) *CA Cancer J Clin.* 65:5–29).

[0005] Non-small cell lung cancer (NSCLC) is the predominant subtype of lung cancer, accounting for approximately 85% of all cases (Molina *et al.* (2008) *Mayo Clin Proc* 83: 584 (94); Howlader *et al.* (2011) *SEE R cancer statistics review, 1975–2011*, National Cancer Institute). NSCLC can be divided into two major histologic types: adenocarcinoma and squamous cell carcinoma (Travis *et al.* (2011) *J Thorac Oncol* 6:244–85). Adenocarcinoma histology accounts for more than half of all NSCLC, while squamous cell histology accounts for approximately 25% (Langer *et al.* (2010) *J Clin Oncol* 28:5311–20). The remaining cases of NSCLC are represented by large cell carcinoma, neuroendocrine tumors, sarcomatoid carcinoma, and poorly differentiated histology.

[0006] The overall 5-year survival rate for advanced disease is 2%–4%, depending on geographic location (Cetin *et al.* (2011) *Clin Epidemiol* 3:139–48). Poor prognostic factors for survival in patients with NSCLC include advanced stage of disease at the time of initial diagnosis,

poor performance status, and a history of unintentional weight loss. More than half of the patients with NSCLC are diagnosed with distant disease, which directly contributes to poor survival prospects.

[0007] Platinum-based regimens remain the standard first-line option for patients with locally advanced or metastatic NSCLC that is not harboring EGFR mutations or ALK gene rearrangements. In particular, for newly diagnosed advanced stage non-squamous NSCLC, the standard of care is a platinum doublet with either cisplatin or carboplatin and a taxane or pemetrexed, with or without bevacizumab. However, current treatment regimens are associated with substantial toxicities (such as febrile neutropenia, myelosuppression, nausea, alopecia, nephropathy, and neuropathy) and are generally poorly tolerated by elderly and poor-performance-status patients. Moreover, the survival benefit conferred by cytotoxic chemotherapy has reached a plateau, with overall response rates of approximately 20% and 1-year survival ranging from 31% to 36% (Schiller *et al.* (2002) *N Engl J Med.* 346: 92–98).

[0008] There are recognized differences in disease characteristics between adenocarcinoma and squamous NSCLC. First, squamous tumors commonly present in the central airways and typically remain localized in the bronchial epithelium (Hirsch *et al.* (2008) *J Thorac Oncol.* 3: 1468–1481), whereas non-squamous tumors are more commonly located in the lung parenchyma distal to the central airways. Evaluation of NSCLC tumor tissues typically reveals cytological differences between the squamous cell type (keratinization, intracellular bridges, and central necrosis) and adenocarcinoma (glandular architecture). In cases where the tumor sample is poorly differentiated or there is limited tissue available, immunohistochemical markers may support the histologic diagnosis. Thyroid transcription factor–1 is infrequently expressed in squamous cells and strongly expressed in adenocarcinoma. In contrast, p63, CK5/6, and 34βE12 are strongly expressed in squamous cell carcinoma and less frequently in adenocarcinoma (Travis *et al.* (2011) *J Thorac Oncol.* 6:244–85).

[0009] Genetic changes that have prognostic and/or predictive significance in NSCLC include mutations in the epidermal growth factor receptor (EGFR), the rearrangement in the anaplastic lymphoma kinase (ALK) genes, and mutations in the GTPase Kras (KRAS) gene. The rates of these mutations differ between squamous cell carcinoma and adenocarcinoma. For example, EGFR kinase domain mutations have been reported in 10%–40% of patients with adenocarcinoma NSCLC but are infrequently observed in patients with squamous NSCLC (Herbst *et al.* (2008) *N Engl J Med.* 359:1367–80). Similarly, the ALK fusion oncogene, recognized as a driver of lung tumorigenesis, is observed in approximately 7% of patients with adenocarcinoma but is very rare in the squamous histology (Herbst *et al.* (2008) *N Engl J Med.* 359:1367–80; Langer *et al.* (2010) *J Clin Oncol* 28: 5311–20). In addition, KRAS mutations are very rare in squamous NSCLC, while they can be observed in up to 30% of cases of adenocarcinoma NSCLC (Travis *et al.* (2011) *J Thorac Oncol.* 6:244–85).

[0010] Genotype-directed therapy has the potential to dramatically improve the balance of benefit and toxicity for selected patients with NSCLC (mainly non-squamous histology) characterized by alterations of driver oncogenes, including sensitizing EGFR mutations and ALK rearrangement. However, these mutations are more prevalent in adenocarcinoma NSCLC and are very rare in squamous NSCLC. Randomized Phase III studies of gefitinib (IPASS), erlotinib (EURTAC), and afatinib (LUX-Lung 3) showed significant improvement of PFS and ORR compared with platinum-doublet chemotherapy (Fukuoka *et al.* (2011) J Clin Oncol. 29: 2866–2874; Rosell *et al.* (2012) Lancet Oncol. 13: 239–246; Yang *et al.* (2012) Lancet Oncol. 13: 539–548). Similarly, the ALK inhibitors crizotinib and ceritinib have demonstrated efficacy in patients with NSCLC positive for ALK rearrangement as defined by fluorescence in situ hybridization (Crino *et al.* (2011) J Clin Oncol. 29: Abstract 7514; Camidge *et al.* (2012) Lancet Oncol. 13: 1011–1019; Shaw *et al.* (2012) European Society of Medical Oncology Meeting. Abstract LBA1 PR; Shaw *et al.* (2014) N Engl J Med. 370: 2537–2539; XALKORI® USPI; ZYKADIA™ USPI).

[0011] Despite progress with new targeted treatments and new chemotherapy combinations, survival rates for advanced NSCLC disease remain low and acquired resistance to targeted agents is a major clinical problem. Accordingly, there is a need in the art for alternative treatment options improve the prognosis of patients with this disease, *e.g.*, methods of treatment that extend survival rate.

[0012] All references cited herein, including patent applications, patent publications, and UniProtKB/Swiss-Prot Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0013] Provided herein are methods and uses of an anti-PD-L1 antibody for treating lung cancer patients. In particular, the methods and uses are based on data from a randomized Phase III clinical study of atezolizumab (TECENTRIQ®) in combination with pemetrexed and a platinum agent (*e.g.*, carboplatin or cisplatin) in treatment naïve individuals (*e.g.*, chemotherapy naïve individuals) with Stage IV non-squamous non-small cell lung cancer (NSCLC). The study demonstrated that initial (first-line) treatment with the combination of TECENTRIQ® (atezolizumab) plus chemotherapy (pemetrexed + carboplatin or pemetrexed + cisplatin) reduced the risk of disease worsening or death (PFS) compared to chemotherapy alone. Additionally, patients who received TECENTRIQ® (atezolizumab) plus chemotherapy (pemetrexed + carboplatin or pemetrexed + cisplatin) demonstrated a numerical improvement in overall survival compared to chemotherapy alone. Safety for the TECENTRIQ and chemotherapy combination appeared consistent with the known safety profile of the individual medicines, and no new safety signals were identified with the combination.

[0014] In one aspect, provided herein are methods of treating an individual having lung cancer, comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an

antimetabolite, and a platinum agent, wherein the treatment extends progression free survival (PFS) of the individual. In some embodiments, the treatment extends overall survival (OS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the treatment increases the PFS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with an antimetabolite (*e.g.*, pemetrexed) and a platinum agent (*e.g.*, carboplatin or cisplatin).

[0015] In another aspect, provided herein are methods of treating an individual having lung cancer, comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an antimetabolite, and a platinum agent, wherein the treatment extends overall survival (OS) of the individual. In some embodiments, overall survival (OS) is measured as the period of time from the start of treatment to death. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months. In some embodiments, the treatment increases the OS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with an antimetabolite (*e.g.*, pemetrexed) and a platinum agent (*e.g.*, carboplatin or cisplatin).

[0016] In some embodiments, the anti-PD-L1 antibody comprises: (a) a heavy chain variable region (VH) that comprises an HVR-H1 comprising an amino acid sequence of GFTFSDSWIH (SEQ ID NO:1), an HVR-2 comprising an amino acid sequence of AWISPYGGSTYYADSVKG (SEQ ID NO:2), and HVR-3 comprising an amino acid sequence of RHWPGGFDY (SEQ ID NO:3), and (b) a light chain variable region (VL) that comprises an HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO:4), an HVR-L2 comprising an amino acid sequence of SASFLYS (SEQ ID NO:5), and an HVR-L3 comprising an amino acid sequence of QQYLYHPAT (SEQ ID NO:6). In some embodiments, the anti-PD-L1 antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO: 7 and a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO: 8. In some embodiments, the anti-PD-L1 antibody is atezolizumab.

[0017] In some embodiments, the antimetabolite is pemetrexed, 5-fluorouracil, 6-mercaptopurine, capecitabine, cytarabine, floxuridine, fludarabine, hydroxycarbamide, or methotrexate. In some embodiments, the antimetabolite is pemetrexed. In some embodiments, the platinum agent is carboplatin. In some embodiments, the platinum agent is cisplatin.

[0018] In some embodiments, the anti-PD-L1 antibody is administered at a dose of 1200 mg, wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve $AUC = 6$ mg/ml/min, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m². In some embodiments, the anti-PD-L1 antibody is administered at a dose of 1200 mg, wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m², and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m². In some embodiments, the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve $AUC = 6$ mg/ml/min on Day 1 of each 21-day cycle for Cycles 1-4. In some embodiments, the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4. In some embodiments, the anti-PD-L1 antibody, antimetabolite, and the platinum agent are administered sequentially on Day 1 of Cycles 1-4. In some embodiments, the anti-PD-L1 antibody is administered prior to the antimetabolite, and wherein the antimetabolite is administered prior to the platinum agent on Day 1 of Cycles 1-4. In some embodiments, the anti-PD-L1 antibody and the antimetabolite are further administered following Cycle 4, wherein the anti-PD-L1 antibody is atezolizumab and is administered at a dose of 1200 mg on Day 1, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the anti-PD-L1 antibody and the antimetabolite are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the anti-PD-L1 antibody is administered prior to the antimetabolite on Day 1 of after Cycle 4.

[0019] In some embodiments, the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve $AUC = 6$ mg/ml/min on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the anti-PD-L1 antibody, the antimetabolite, and the platinum

agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the anti-PD-L1 antibody, antimetabolite, and the platinum agent are administered sequentially on Day 1 of Cycles 1-6. In some embodiments, the anti-PD-L1 antibody is administered prior to the antimetabolite, and wherein the antimetabolite is administered prior to the platinum agent on Day 1 of Cycles 1-6. In some embodiments, the anti-PD-L1 antibody and the antimetabolite are further administered following Cycle 6, wherein the anti-PD-L1 antibody is atezolizumab and is administered at a dose of 1200 mg on Day 1, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the anti-PD-L1 antibody and the antimetabolite are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the anti-PD-L1 antibody is administered prior to the antimetabolite on Day 1 of each 21-day cycle for every cycle after Cycle 6.

[0020] In some embodiments, the anti-PD-L1 antibody, the platinum agent, and the antimetabolite inhibitor are each administered intravenously. In some embodiments, the lung cancer is non-small cell lung cancer (NSCLC). In some embodiments, the NSCLC is Stage IV non-squamous NSCLC. In some embodiments, the individual is treatment-naïve for Stage IV non-squamous NSCLC. In some embodiments, the individual is chemotherapy-naïve for Stage IV non-squamous NSCLC. In some embodiments, the individual is Asian or of Asian descent. In some embodiments, the individual is at least 65 years old. In some embodiments, the individual is a never smoker. In some embodiments, the individual is PD-L1 high. In some embodiments, the individual is PD-L1 negative. In some embodiments, the individual is human.

[0021] In another aspect, provided herein are methods of treating an individual having Stage IV non-squamous non-small cell lung cancer (NSCLC), comprising administering to the individual an effective amount of atezolizumab, pemetrexed, and carboplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min, wherein the treatment extends progression free survival (PFS). In some embodiments, the treatment extends overall survival (OS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the treatment increases the PFS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 months (including any range in between these values), as compared to an individual having lung

cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with pemetrexed and carboplatin. In some embodiments, treatment extends the overall survival (OS) of the individual. In some embodiments, overall survival (OS) is measured as the period of time from the start of treatment to death. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months. In some embodiments, the treatment increases the OS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with pemetrexed and carboplatin. In some embodiments, the atezolizumab, pemetrexed, and carboplatin are administered in four 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 4; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-4, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4, and carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle of Cycles 1-4, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 4 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab, pemetrexed, and carboplatin are administered sequentially on Day 1 of Cycles 1-4. In some embodiments, the atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to carboplatin on Day 1 of Cycles 1-4. In some embodiments, the atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab, pemetrexed, and carboplatin are administered in six 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 6, wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-6, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6, and carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle of Cycles 1-6, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 6 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the atezolizumab, pemetrexed, and carboplatin are administered sequentially on Day 1 of Cycles 1-6. In some embodiments, the atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to carboplatin on Day 1 of Cycles 1-6. In some

embodiments, the atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the atezolizumab, the pemetrexed, and the carboplatin are each administered intravenously.

[0022] In another aspect, provided herein are methods of treating an individual having Stage IV non-squamous non-small cell lung cancer (NSCLC), comprising administering to the individual an effective amount of atezolizumab, pemetrexed, and cisplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the cisplatin is administered at a dose of 75 mg/m², wherein the treatment extends progression free survival (PFS) of the individual. In some embodiments, the treatment extends overall survival (OS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the treatment increases the PFS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with pemetrexed and cisplatin. In some embodiments, treatment extends the overall survival (OS) of the individual. In some embodiments, overall survival (OS) is measured as the period of time from the start of treatment to death. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months. In some embodiments, the treatment increases the OS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with pemetrexed and cisplatin. In some embodiments, the atezolizumab, pemetrexed, and cisplatin are administered in four 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 4; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-4, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4, and cisplatin is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle of Cycles 1-4, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 4 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab, pemetrexed, and cisplatin are administered sequentially on Day 1 of Cycles 1-4. In some embodiments, the

atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to cisplatin on Day 1 of Cycles 1-4. In some embodiments, the atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 4. In some embodiments, the atezolizumab, pemetrexed, and cisplatin are administered in six 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 6; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-6, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6, and cisplatin is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle of Cycles 1-6, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 6 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the atezolizumab, pemetrexed, and cisplatin are administered sequentially on Day 1 of Cycles 1-6. In some embodiments, the atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to cisplatin on Day 1 of Cycles 1-6. In some embodiments, the wherein atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 6. In some embodiments, the atezolizumab, the pemetrexed, and the cisplatin are each administered intravenously.

[0023] In some embodiments, the individual is treatment-naïve for Stage IV non-squamous NSCLC. In some embodiments, the individual is chemotherapy-naïve for Stage IV non-squamous NSCLC. In some embodiments, the individual is Asian or of Asian descent. In some embodiments, the individual is at least 65 years old. In some embodiments, the individual is a never smoker. In some embodiments, the individual is PD-L1 high. In some embodiments, the individual is PD-L1 negative. In some embodiments, the individual is human.

[0024] In another aspect, provided are kits comprising an anti-PD-L1 antibody for use in combination with an antimetabolite and platinum agent for treating an individual having lung cancer according to a method disclosed herein. In some embodiments, provided are kits comprising atezolizumab for use in combination with pemetrexed and carboplatin for treating an individual having lung cancer according to a method disclosed herein. In some embodiments, provided are kits comprising atezolizumab for use in combination with pemetrexed and cisplatin for treating an individual having lung cancer according to a method disclosed herein.

[0025] In another aspect, provided herein is a composition comprising an anti-PD-L1 antibody for use in a method of treating lung cancer in an individual, the method comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an antimetabolite, and a platinum agent, wherein the treatment extends progression free survival (PFS) of the individual. In some

embodiments, the treatment extends the overall survival (OS) of the individual. In some embodiments, the composition comprising the anti-PD-L1 antibody is for use according to a method disclosed herein.

[0026] In another aspect, provided herein is a composition comprising atezolizumab for use in a method of treating Stage IV non-squamous non-small cell lung cancer (NSCLC), the method comprising administering to the individual an effective amount of atezolizumab, pemetrexed and carboplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min, and wherein the treatment extends progression free survival (PFS) of the individual. In some embodiments, the treatment extends the overall survival (OS) of the individual. In some embodiments, the composition is for use according to a method disclosed herein.

[0027] In another aspect, provided herein is a composition comprising atezolizumab for use in a method of treating Stage IV non-squamous non-small cell lung cancer (NSCLC), the method comprising administering to the individual an effective amount of atezolizumab, pemetrexed and cisplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the cisplatin is administered at a dose of 75 mg/m², and wherein the treatment extends progression free survival (PFS) of the individual. In some embodiments, the treatment extends the overall survival (OS) of the individual. In some embodiments, the composition is for use according to a method disclosed herein.

[0028] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art. These and other embodiments of the invention are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** provides a schematic of the study design of the clinical trial described in Example 1. 578 patients were enrolled. Arm A included 292 patients, and Arm B included 286 patients. Patients in Arm A received treatment until progressive disease (PD) or loss of clinical benefit, and patients in Arm B received treatment until PD.

[0030] **FIG. 2** provides a Kaplan-Meier Plot of progression-free survival (PFS) of patients in Arm A (atezolizumab + pemetrexed + carboplatin or cisplatin) vs. Arm B (pemetrexed + carboplatin or cisplatin).

[0031] **FIG. 3** provides a Kaplan-Meier Plot of overall survival (OS) of patients in Arm A (atezolizumab + pemetrexed + carboplatin or cisplatin) vs. Arm B (pemetrexed + carboplatin or cisplatin).

[0032] FIG. 4 provides a comparison of confirmed overall response rate (ORR) in patients in Arm A. vs. Arm B. (CR = complete response; CR/PR = complete response/partial response; SD = stable disease; PD = progressive disease.) ORR was assessed according to RECIST v1.1 criteria.

[0033] FIG. 5A provides a Forest Plot showing subgroup analyses of PFS in patients with various baseline risk factors in Arm A (APP) vs. Arm B (PP). (APP = atezolizumab + pemetrexed + carboplatin or cisplatin; PP = pemetrexed + carboplatin or cisplatin.)

[0034] FIG. 5B provides a Forest Plot showing subgroup analyses of PFS in patients with various baseline risk factors in Arm A (APP) vs. Arm B (PP). (APP = atezolizumab + pemetrexed + carboplatin or cisplatin; PP = pemetrexed + carboplatin or cisplatin.)

[0035] FIG. 6 provides a Forest Plot showing subgroup analyses of OS in patients with various baseline risk factors in Arm A (APP) vs. Arm B (PP). (APP = atezolizumab + pemetrexed + carboplatin or cisplatin; PP = pemetrexed + carboplatin or cisplatin.)

[0036] FIG. 7A provides a Kaplan-Meier Plot of progression-free survival (PFS) of “PD-L1 high” patients in Arm A (atezolizumab + pemetrexed + carboplatin or cisplatin) vs. Arm B (pemetrexed + carboplatin or cisplatin).

[0037] FIG. 7B provides a Kaplan-Meier Plot of progression-free survival (PFS) of “PD-L1 low” patients in Arm A (atezolizumab + pemetrexed + carboplatin or cisplatin) vs. Arm B (pemetrexed + carboplatin or cisplatin).

[0038] FIG. 7C Plot of progression-free survival (PFS) of “PD-L1 negative” patients in Arm A (atezolizumab + pemetrexed + carboplatin or cisplatin) vs. Arm B (pemetrexed + carboplatin or cisplatin).

DETAILED DESCRIPTION

I. Definitions

[0039] Before describing the invention in detail, it is to be understood that this invention is not limited to particular compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0040] As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a molecule” optionally includes a combination of two or more such molecules, and the like.

[0041] The term “about” as used herein 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) embodiments that are directed to that value or parameter per se.

[0042] It is understood that aspects and embodiments of the invention described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

[0043] The term “PD-1 axis binding antagonist” refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with either one or more of its binding partner, so as to remove T-cell dysfunction resulting from signaling on the PD-1 signaling axis – with a result being to restore or enhance T-cell function (*e.g.*, proliferation, cytokine production, target cell killing). As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PD-L1 binding antagonist and a PD-L2 binding antagonist.

[0044] The term “PD-1 binding antagonist” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1, PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to one or more of its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. Specific examples of PD-1 binding antagonists are provided *infra*.

[0045] The term “PD-L1 binding antagonist” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1, B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, the PD-L1 binding antagonists include anti-PD-L1 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1, B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L1 so as to render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. Specific examples of PD-L1 binding antagonists are provided *infra*.

[0046] The term “PD-L2 binding antagonist” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (*e.g.*, enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

[0047] “Sustained response” refers to the sustained effect on reducing tumor growth after cessation of a treatment. For example, the tumor size may remain to be the same or smaller as compared to the size at the beginning of the administration phase. In some embodiments, the sustained response has a duration at least the same as the treatment duration, at least 1.5X, 2.0X, 2.5X, or 3.0X length of the treatment duration.

[0048] The term “pharmaceutical formulation” refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. “Pharmaceutically acceptable” excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0049] As used herein, the term “treatment” refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully “treated” if one or more symptoms associated with cancer are mitigated or eliminated, including, but are not limited to, reducing the proliferation of (or destroying) cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0050] As used herein, “delaying progression of a disease” means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease (such as cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that

the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

[0051] An “effective amount” is at least the minimum amount required to effect a measurable improvement or prevention of a particular disorder. An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (*i.e.*, slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (*i.e.*, slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an “effective amount” may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0052] As used herein, “in conjunction with” refers to administration of one treatment modality in addition to another treatment modality. As such, “in conjunction with” refers to administration of one treatment modality before, during, or after administration of the other treatment modality to the individual.

[0053] A “disorder” is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

[0054] The terms “cell proliferative disorder” and “proliferative disorder” refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

[0055] “Tumor,” as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer”, “cancerous”, “cell proliferative disorder”, “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein.

[0056] The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include, but not limited to, squamous cell cancer (*e.g.*, epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo maligna melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin’s lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenstrom’s Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phakomatoses, edema (such as that associated with brain tumors), Meigs’ syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by the antibodies of the invention include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkins lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi’s sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, glioblastoma, neuroblastomas, melanoma, breast carcinoma, gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma.

[0057] The term “cytotoxic agent” as used herein refers to any agent that is detrimental to cells (*e.g.*, causes cell death, inhibits proliferation, or otherwise hinders a cellular function). Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Exemplary cytotoxic agents can be selected from anti-microtubule agents, platinum coordination complexes, alkylating agents, antibiotic agents, topoisomerase II inhibitors, antimetabolites, topoisomerase I inhibitors, hormones and hormonal analogues, signal transduction pathway inhibitors, non-receptor tyrosine kinase angiogenesis inhibitors, immunotherapeutic agents, proapoptotic agents, inhibitors of LDH-A, inhibitors of fatty acid biosynthesis, cell cycle signaling inhibitors, HDAC inhibitors, proteasome inhibitors, and inhibitors of cancer metabolism. In one embodiment the cytotoxic agent is a taxane. In one embodiment the taxane is paclitaxel or docetaxel. In one embodiment the cytotoxic agent is a platinum agent. In one embodiment the cytotoxic agent is an antagonist of EGFR. In one embodiment the antagonist of EGFR is N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (*e.g.*, erlotinib). In one embodiment the cytotoxic agent is a RAF inhibitor. In one embodiment, the RAF inhibitor is a BRAF and/or CRAF inhibitor. In one embodiment the RAF inhibitor is vemurafenib. In one embodiment the cytotoxic agent is a PI3K inhibitor.

[0058] “Chemotherapeutic agent” includes compounds useful in the treatment of cancer. Examples of chemotherapeutic agents include erlotinib (TARCEVA®, Genentech/OSI Pharm.), bortezomib (VELCADE®, Millennium Pharm.), disulfiram, epigallocatechin gallate, salinosporamide A, carfilzomib, 17-AAG (geldanamycin), radicicol, lactate dehydrogenase A (LDH-A), fulvestrant (FASLODEX®, AstraZeneca), sunitib (SUTENT®, Pfizer/Sugen), letrozole (FEMARA®, Novartis), imatinib mesylate (GLEEVEC®, Novartis), finasunate (VATALANIB®, Novartis), oxaliplatin (ELOXATIN®, Sanofi), 5-FU (5-fluorouracil), leucovorin, Rapamycin (Sirolimus, RAPAMUNE®, Wyeth), Lapatinib (TYKERB®, GSK572016, Glaxo Smith Kline), Lonafamib (SCH 66336), sorafenib (NEXAVAR®, Bayer Labs), gefitinib (IRESSA®, AstraZeneca), AG1478, alkylating agents such as thiotepa and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including topotecan and irinotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogs); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); adrenocorticosteroids (including prednisone and prednisolone); cyproterone acetate; 5 α -reductases including finasteride and

dutasteride); vorinostat, romidepsin, panobinostat, valproic acid, mocetinostat dolastatin; aldesleukin, talc duocarmycin (including the synthetic analogs, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlomaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin γ II and calicheamicin ω II (Angew Chem. Intl. Ed. Engl. 1994 33:183-186); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® (doxorubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogs such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanol, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamnol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepe; taxoids, *e.g.*, TAXOL (paclitaxel; Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE® (Cremophor-free), albumin-engineered nanoparticle formulations of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERE® (docetaxel, doxetaxel; Sanofi-Aventis); chlorambucil; GEMZAR® (gemcitabine); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; etoposide (VP-16); ifosfamide;

mitoxantrone; vincristine; NAVELBINE® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; capecitabine (XELODA®); ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; and pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0059] Chemotherapeutic agent also includes (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX®; tamoxifen citrate), raloxifene, droloxifene, idoxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® (toremifene citrate); (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® (megestrol acetate), AROMASIN® (exemestane; Pfizer), formestane, fadrozole, RIVISOR® (vorozole), FEMARA® (letrozole; Novartis), and ARIMIDEX® (anastrozole; AstraZeneca); (iii) anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide and goserelin; buserelin, triptorelin, medroxyprogesterone acetate, diethylstilbestrol, premarin, fluoxymesterone, all transretinoic acid, fenretinide, as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, particularly those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Ral and H-Ras; (vii) ribozymes such as VEGF expression inhibitors (*e.g.*, ANGIOZYME®) and HER2 expression inhibitors; (viii) vaccines such as gene therapy vaccines, for example, ALLOVECTIN®, LEUVECTIN®, and VAXID®; PROLEUKIN®, rIL-2; a topoisomerase 1 inhibitor such as LURTOTECAN®; ABARELIX® rmRH; and (ix) pharmaceutically acceptable salts, acids and derivatives of any of the above.

[0060] Chemotherapeutic agent also includes antibodies such as alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixa), and the antibody drug conjugate, gemtuzumab ozogamicin (MYLOTARG®, Wyeth). Additional humanized monoclonal antibodies with therapeutic potential as agents in combination with the compounds of the invention include: apolizumab, aselizumab, atlizumab, bapineuzumab, bivatumumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab,

rovelizumab, ruplizumab, sibrotuzumab, siplizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucosituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories) which is a recombinant exclusively human-sequence, full-length IgG1 λ antibody genetically modified to recognize interleukin-12 p40 protein.

[0061] Chemotherapeutic agent also includes “EGFR inhibitors,” which refers to compounds that bind to or otherwise interact directly with EGFR and prevent or reduce its signaling activity, and is alternatively referred to as an “EGFR antagonist.” Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAb 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (*see*, US Patent No. 4,943, 533, Mendelsohn *et al.*) and variants thereof, such as chimerized 225 (C225 or Cetuximab; ERBUTIX®) and reshaped human 225 (H225) (*see*, WO 96/40210, Imclone Systems Inc.); IMC-11F8, a fully human, EGFR-targeted antibody (Imclone); antibodies that bind type II mutant EGFR (US Patent No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in US Patent No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF or Panitumumab (*see* WO98/50433, Abgenix/Amgen); EMD 55900 (Stragliotto *et al.* Eur. J. Cancer 32A:636-640 (1996)); EMD7200 (matuzumab) a humanized EGFR antibody directed against EGFR that competes with both EGF and TGF- α for EGFR binding (EMD/Merck); human EGFR antibody, HuMax-EGFR (GenMab); fully human antibodies known as E1.1, E2.4, E2.5, E6.2, E6.4, E2.11, E6.3 and E7.6.3 and described in US 6,235,883; MDX-447 (Medarex Inc); and mAb 806 or humanized mAb 806 (Johns *et al.*, J. Biol. Chem. 279(29):30375-30384 (2004)). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (*see, e.g.*, EP659439A2, Merck Patent GmbH). EGFR antagonists include small molecules such as compounds described in US Patent Nos: 5,616,582, 5,457,105, 5,475,001, 5,654,307, 5,679,683, 6,084,095, 6,265,410, 6,455,534, 6,521,620, 6,596,726, 6,713,484, 5,770,599, 6,140,332, 5,866,572, 6,399,602, 6,344,459, 6,602,863, 6,391,874, 6,344,455, 5,760,041, 6,002,008, and 5,747,498, as well as the following PCT publications: WO98/14451, WO98/50038, WO99/09016, and WO99/24037. Particular small molecule EGFR antagonists include OSI-774 (CP-358774, erlotinib, TARCEVA® Genentech/OSI Pharmaceuticals); PD 183805 (CI 1033, 2-propenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[3-(4-morpholinyl)propoxy]-6-quinazolyl]-, dihydrochloride, Pfizer Inc.); ZD1839, gefitinib (IRESSA®) 4-(3'-Chloro-4'-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline, AstraZeneca); ZM 105180 ((6-amino-4-(3-methylphenyl-amino)-quinazoline, Zeneca); BIBX-1382 (N8-(3-chloro-4-fluoro-phenyl)-N2-(1-methyl-piperidin-4-yl)-pyrimido[5,4-d]pyrimidine-2,8-diamine, Boehringer Ingelheim); PKI-166 ((R)-4-[4-[(1-phenylethyl)amino]-1H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol); (R)-6-(4-

hydroxyphenyl)-4-[(1-phenylethyl)amino]-7H-pyrrolo[2,3-d]pyrimidine); CL-387785 (N-[4-[(3-bromophenyl)amino]-6-quinazoliny]-2-butyramide); EKB-569 (N-[4-[(3-chloro-4-fluorophenyl)amino]-3-cyano-7-ethoxy-6-quinoliny]-4-(dimethylamino)-2-butenamide) (Wyeth); AG1478 (Pfizer); AG1571 (SU 5271; Pfizer); dual EGFR/HER2 tyrosine kinase inhibitors such as lapatinib (TYKERB®, GSK572016 or N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6[[[2-methylsulfonyl]ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine).

[0062] Chemotherapeutic agents also include “tyrosine kinase inhibitors” including the EGFR-targeted drugs noted in the preceding paragraph; small molecule HER2 tyrosine kinase inhibitor such as TAK165 available from Takeda; CP-724,714, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase (Pfizer and OSI); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase I inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035, 4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (e.g. those that bind to HER-encoding nucleic acid); quinoxalines (US Patent No. 5,804,396); tryphostins (US Patent No. 5,804,396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering AG); pan-HER inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); imatinib mesylate (GLEEVEC®); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxinib (Pfizer); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C11 (Imclone), rapamycin (sirolimus, RAPAMUNE®); or as described in any of the following patent publications: US Patent No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[0063] Chemotherapeutic agents also include dexamethasone, interferons, colchicine, metoprine, cyclosporine, amphotericin, metronidazole, alemtuzumab, alitretinoin, allopurinol, amifostine, arsenic trioxide, asparaginase, BCG live, bevacuzimab, bexarotene, cladribine, clofarabine, darbepoetin alfa,

denileukin, dexrazoxane, epoetin alfa, elotinib, filgrastim, histrelin acetate, ibritumomab, interferon alfa-2a, interferon alfa-2b, lenalidomide, levamisole, mesna, methoxsalen, nandrolone, nelarabine, nofetumomab, oprelvekin, palifermin, pamidronate, pegademase, pegaspargase, pegfilgrastim, pemetrexed disodium, plicamycin, porfimer sodium, quinacrine, rasburicase, sargramostim, temozolomide, VM-26, 6-TG, toremifene, tretinoin, ATRA, valrubicin, zoledronate, and zoledronic acid, and pharmaceutically acceptable salts thereof.

[0064] Chemotherapeutic agents also include hydrocortisone, hydrocortisone acetate, cortisone acetate, tixocortol pivalate, triamcinolone acetonide, triamcinolone alcohol, mometasone, amcinonide, budesonide, desonide, fluocinonide, fluocinolone acetonide, betamethasone, betamethasone sodium phosphate, dexamethasone, dexamethasone sodium phosphate, fluocortolone, hydrocortisone-17-butyrate, hydrocortisone-17-valerate, aclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, prednicarbate, clobetasone-17-butyrate, clobetasol-17-propionate, fluocortolone caproate, fluocortolone pivalate and fluprednidene acetate; immune selective anti-inflammatory peptides (ImSAIDs) such as phenylalanine-glutamine-glycine (FEG) and its D-isomeric form (feG) (IMULAN BioTherapeutics, LLC); anti-rheumatic drugs such as azathioprine, ciclosporin (cyclosporine A), D-penicillamine, gold salts, hydroxychloroquine, leflunomide, minocycline, sulfasalazine, tumor necrosis factor alpha (TNF α) blockers such as etanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), certolizumab pegol (Cimzia), golimumab (Simponi), Interleukin 1 (IL-1) blockers such as anakinra (Kineret), T cell costimulation blockers such as abatacept (Orencia), Interleukin 6 (IL-6) blockers such as tocilizumab (ACTEMERA®); Interleukin 13 (IL-13) blockers such as lebrikizumab; Interferon alpha (IFN) blockers such as Rontalizumab; Beta 7 integrin blockers such as rhuMAb Beta7; IgE pathway blockers such as Anti-M1 prime; Secreted homotrimeric LTA β and membrane bound heterotrimer LTA β 2 blockers such as Anti-lymphotoxin alpha (LTA); radioactive isotopes (*e.g.*, At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu); miscellaneous investigational agents such as thioplatin, PS-341, phenylbutyrate, ET-18-OCH $_3$, or farnesyl transferase inhibitors (L-739749, L-744832); polyphenols such as quercetin, resveratrol, piceatannol, epigallocatechin gallate, theaflavins, flavanols, procyanidins, betulinic acid and derivatives thereof; autophagy inhibitors such as chloroquine; delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; acetylcamptothecin, scoplectin, and 9-aminocamptothecin); podophyllotoxin; tegafur (UFTORAL®); bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine; perifosine, COX-2 inhibitor (*e.g.*, celecoxib or etoricoxib), proteasome inhibitor (*e.g.*, PS341); CCI-779; tipifarnib (R11577); orafenib, ABT510;

Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASARTM); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovorin.

[0065] Chemotherapeutic agents also include non-steroidal anti-inflammatory drugs with analgesic, antipyretic and anti-inflammatory effects. NSAIDs include non-selective inhibitors of the enzyme cyclooxygenase. Specific examples of NSAIDs include aspirin, propionic acid derivatives such as ibuprofen, fenoprofen, ketoprofen, flurbiprofen, oxaprozin and naproxen, acetic acid derivatives such as indomethacin, sulindac, etodolac, diclofenac, enolic acid derivatives such as piroxicam, meloxicam, tenoxicam, droxicam, lornoxicam and isoxicam, fenamic acid derivatives such as mefenamic acid, meclofenamic acid, flufenamic acid, tolfenamic acid, and COX-2 inhibitors such as celecoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, rofecoxib, and valdecoxib. NSAIDs can be indicated for the symptomatic relief of conditions such as rheumatoid arthritis, osteoarthritis, inflammatory arthropathies, ankylosing spondylitis, psoriatic arthritis, Reiter's syndrome, acute gout, dysmenorrhoea, metastatic bone pain, headache and migraine, postoperative pain, mild-to-moderate pain due to inflammation and tissue injury, pyrexia, ileus, and renal colic.

[0066] A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell either in vitro or in vivo. In one embodiment, growth inhibitory agent is growth inhibitory antibody that prevents or reduces proliferation of a cell expressing an antigen to which the antibody binds. In another embodiment, the growth inhibitory agent may be one which significantly reduces the percentage of cells in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami *et al.* (W.B. Saunders, Philadelphia, 1995), *e.g.*, p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0067] By “radiation therapy” is meant the use of directed gamma rays or beta rays to induce sufficient damage to a cell so as to limit its ability to function normally or to destroy the cell altogether. It will be appreciated that there will be many ways known in the art to determine the dosage and duration of treatment. Typical treatments are given as a one-time administration and typical dosages range from 10 to 200 units (Grays) per day.

[0068] A “subject” or an “individual” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[0069] The term “antibody” herein is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired biological activity.

[0070] An “isolated” antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of, for example, a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using, for example, Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody’s natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0071] “Native antibodies” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0072] The term “constant domain” refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the

variable domain, which contains the antigen binding site. The constant domain contains the CH1, CH2 and CH3 domains (collectively, CH) of the heavy chain and the CHL (or CL) domain of the light chain.

[0073] The “variable region” or “variable domain” of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domain of the heavy chain may be referred to as “VH.” The variable domain of the light chain may be referred to as “VL.” These domains are generally the most variable parts of an antibody and contain the antigen-binding sites.

[0074] The term “variable” refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (*see Kabat et al.*, Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0075] The “light chains” of antibodies (immunoglobulins) from any mammalian species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains.

[0076] The term IgG “isotype” or “subclass” as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions.

[0077] Depending on the amino acid sequences of the constant domains of their heavy chains, antibodies (immunoglobulins) can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, γ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.* Cellular and Mol. Immunology, 4th ed. (W.B. Saunders, Co., 2000). An antibody may be part of a larger fusion molecule, formed by covalent or non-covalent association of the antibody with one or more other proteins or peptides.

[0078] The terms “full length antibody,” “intact antibody” and “whole antibody” are used herein interchangeably to refer to an antibody in its substantially intact form, not antibody fragments as defined below. The terms particularly refer to an antibody with heavy chains that contain an Fc region.

[0079] A “naked antibody” for the purposes herein is an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0080] “Antibody fragments” comprise a portion of an intact antibody, preferably comprising the antigen binding region thereof. In some embodiments, the antibody fragment described herein is an antigen-binding fragment. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0081] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0082] “Fv” is the minimum antibody fragment which contains a complete antigen-binding site. In one embodiment, a two-chain Fv species consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv (scFv) species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a “dimeric” structure analogous to that in a two-chain Fv species. It is in this configuration that the three HVRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six HVRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0083] The Fab fragment contains the heavy- and light-chain variable domains and also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0084] “Single-chain Fv” or “scFv” antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, *see, e.g.,* Pluckthün,

in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York, 1994), pp. 269-315.

[0085] The term “diabodies” refers to antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies may be bivalent or bispecific. Diabodies are described more fully in, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, *Nat. Med.* 9:129-134 (2003); and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, *Nat. Med.* 9:129-134 (2003).

[0086] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *e.g.*, the individual antibodies comprising the population are identical except for possible mutations, *e.g.*, naturally occurring mutations, that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies. In certain embodiments, such a monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones, or recombinant DNA clones. It should be understood that a selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins.

[0087] The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein, *Nature*, 256:495-97 (1975); Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring

Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (*see, e.g.*, Clackson *et al.*, *Nature*, 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (*see, e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0088] The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (*see, e.g.*, U.S. Pat. No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984)). Chimeric antibodies include PRIMATTZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with the antigen of interest.

[0089] “Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from a HVR of the recipient are replaced by residues from a HVR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin, and all or substantially all of the FRs are those

of a human immunoglobulin sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see, e.g.*, Jones *et al.*, Nature 321:522-525 (1986); Riechmann *et al.*, Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). *See also, e.g.*, Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1:105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0090] A “human antibody” is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks *et al.*, J. Mol. Biol., 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner *et al.*, J. Immunol., 147(1):86-95 (1991). *See also* van Dijk and van de Winkel, Curr Opin Pharmacol. 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (*see, e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). *See also*, for example, Li *et al.*, Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0091] A “species-dependent antibody” is one which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody “binds specifically” to a human antigen (*e.g.*, has a binding affinity (Kd) value of no more than about 1×10^{-7} M, preferably no more than about 1×10^{-8} M and preferably no more than about 1×10^{-9} M) but has a binding affinity for a homologue of the antigen from a second nonhuman mammalian species which is at least about 50 fold, or at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the human antigen. The species-dependent antibody can be any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

[0092] The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. *See, e.g.*,

Xu *et al.*, Immunity 13:37-45 (2000); Johnson and Wu, in Methods in Molecular Biology 248:1-25 (Lo, ed., Human Press, Totowa, N.J., 2003). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. *See, e.g.*, Hamers-Casterman *et al.*, Nature 363:446-448 (1993); Sheriff *et al.*, Nature Struct. Biol. 3:733-736 (1996).

[0093] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat Numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia Numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0094] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra*, for each of these definitions.

[0095] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al.*, *supra*, for each of these definitions.

[0096] "Framework" or "FR" residues are those variable domain residues other than the HVR residues as herein defined.

[0097] The term "variable domain residue numbering as in Kabat" or "amino acid position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy chain

variable domains or light chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.*, residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

[0098] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g.*, Kabat *et al.*, Sequences of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g.*, the EU index reported in Kabat *et al.*, supra). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody

[0099] The expression “linear antibodies” refers to the antibodies described in Zapata *et al.* (1995 Protein Eng. 8(10):1057-1062). Briefly, these antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

[0100] As use herein, the term “binds”, “specifically binds to” or is “specific for” refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that binds to or specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 100\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, or $\leq 0.1\text{ nM}$. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0101] The term “sample,” as used herein, refers to a composition that is obtained or derived from a subject and/or individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase “disease sample” and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the

cellular and/or molecular entity that is to be characterized. Samples include, but are not limited to, primary or cultured cells or cell lines, cell supernatants, cell lysates, platelets, serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, blood-derived cells, urine, cerebrospinal fluid, saliva, sputum, tears, perspiration, mucus, tumor lysates, and tissue culture medium, tissue extracts such as homogenized tissue, tumor tissue, cellular extracts, and combinations thereof.

[0102] By “tissue sample” or “cell sample” is meant a collection of similar cells obtained from a tissue of a subject or individual. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, and/or aspirate; blood or any blood constituents such as plasma; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the subject. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like.

[0103] A “reference sample”, “reference cell”, “reference tissue”, “control sample”, “control cell”, or “control tissue”, as used herein, refers to a sample, cell, tissue, standard, or level that is used for comparison purposes. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissue or cells) of the same subject or individual. For example, healthy and/or non-diseased cells or tissue adjacent to the diseased cells or tissue (*e.g.*, cells or tissue adjacent to a tumor). In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or individual. In yet another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy and/or non-diseased part of the body (*e.g.*, tissues or cells) of an individual who is not the subject or individual. In even another embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from an untreated tissue and/or cell of the body of an individual who is not the subject or individual.

[0104] An “effective response” of a patient or a patient’s “responsiveness” to treatment with a medicament and similar wording refers to the clinical or therapeutic benefit imparted to a patient at risk for, or suffering from, a disease or disorder, such as cancer. In one embodiment, such benefit includes any one or more of: extending survival (including overall survival and progression free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer.

[0105] A patient who “does not have an effective response” to treatment refers to a patient who does not have any one of extending survival (including overall survival and progression free survival);

resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer.

[0106] A “functional Fc region” possesses an “effector function” of a native sequence Fc region. Exemplary “effector functions” include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (*e.g.*, B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.*, an antibody variable domain) and can be assessed using various assays as disclosed, for example, in definitions herein.

[0107] “Human effector cells” refer to leukocytes that express one or more FcRs and perform effector functions. In certain embodiments, the cells express at least FcγRIII and perform ADCC effector function(s). Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells, and neutrophils. The effector cells may be isolated from a native source, *e.g.*, from blood.

[0108] A cancer or biological sample which “has human effector cells” is one which, in a diagnostic test, has human effector cells present in the sample (*e.g.*, infiltrating human effector cells).

[0109] A cancer or biological sample which “has FcR-expressing cells” is one which, in a diagnostic test, has FcR-expressing present in the sample (*e.g.*, infiltrating FcR-expressing cells). In some embodiments, FcR is FcγR. In some embodiments, FcR is an activating FcγR.

II. Overview

[0110] Provided herein is a method for treating or delaying progression of lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody, such as atezolizumab), an antimetabolite (*e.g.*, pemetrexed), and a platinum agent (*e.g.*, carboplatin or cisplatin). Also provided herein is a method of enhancing immune function in an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) comprising administering to the individual an effective amount of a PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody, such as atezolizumab) an antimetabolite (*e.g.*, pemetrexed), and a platinum agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the treatment extends the progression free survival (PFS) and/or the overall survival (OS) of the individual. In some embodiments, the treatment extends the progression free survival (PFS) and/or the overall survival (OS) of the individual, as compared to a treatment comprising administration of an antimetabolite (*e.g.*, pemetrexed) and a platinum agent (*e.g.*, carboplatin or cisplatin).

[0111] In some embodiments, the method comprises treating an individual having Stage IV lung cancer, *e.g.*, Stage IV non-squamous NSCLC, by administering to the individual atezolizumab in combination with pemetrexed and carboplatin, wherein the administering comprises an induction

phase and a maintenance phase, wherein the induction phase comprises administering atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the carboplatin at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle for Cycles 1-4; and wherein the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4; wherein the individual is treatment-naïve and has Stage IV non-squamous non-small cell lung cancer (NSCLC); and wherein the administering extends the progression free survival (PFS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the administering extends the overall survival (OS) of the individual. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months.

[0112] In some embodiments, the method comprises treating an individual having Stage IV lung cancer, *e.g.*, Stage IV non-squamous NSCLC, by administering to the individual atezolizumab in combination with pemetrexed and cisplatin, wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the cisplatin at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4; and wherein the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4; wherein the individual is treatment-naïve and has Stage IV non-squamous non-small cell lung cancer (NSCLC); and wherein the administering extends the progression free survival (PFS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the administering extends the overall survival (OS) of the individual. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months.

[0113] In some embodiments, the method comprises treating an individual having Stage IV lung cancer, *e.g.*, Stage IV non-squamous NSCLC, by administering to the individual atezolizumab in combination with pemetrexed and carboplatin, wherein the administering comprises an induction

phase and a maintenance phase, wherein the induction phase comprises administering atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the carboplatin at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle for Cycles 1-6; and wherein the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6; wherein the individual is treatment-naïve and has Stage IV non-squamous non-small cell lung cancer (NSCLC); and wherein the administering extends the progression free survival (PFS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the administering extends the overall survival (OS) of the individual. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months.

[0114] In some embodiments, the method comprises treating an individual having Stage IV non-small cell lung cancer (NSCLC), *e.g.*, Stage IV non-squamous NSCLC, by administering to the individual atezolizumab in combination with pemetrexed and cisplatin, wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the cisplatin at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6; and wherein the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6; wherein the individual is treatment-naïve and has Stage IV non-squamous non-small cell lung cancer (NSCLC); and wherein the administering extends the progression free survival (PFS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the administering extends the overall survival (OS) of the individual. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months.

III. PD-1 Axis Binding Antagonists

[0115] For example, a PD-1 axis binding antagonist includes a PD-1 binding antagonist, a PDL1 binding antagonist and a PDL2 binding antagonist. Alternative names for “PD-1” include CD279

and SLEB2. Alternative names for “PDL1” include B7-H1, B7-4, CD274, and B7-H. Alternative names for “PDL2” include B7-DC, Btdc, and CD273. In some embodiments, PD-1, PDL1, and PDL2 are human PD-1, PDL1 and PDL2.

[0116] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partner(s). In a specific aspect the PD-1 ligand binding partners are PDL1 and/or PDL2. In another embodiment, a PDL1 binding antagonist is a molecule that inhibits the binding of PDL1 to its binding partner(s). In a specific aspect, PDL1 binding partner(s) are PD-1 and/or B7-1. In another embodiment, the PDL2 binding antagonist is a molecule that inhibits the binding of PDL2 to its binding partner(s). In a specific aspect, a PDL2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0117] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody).

[0118] In some embodiments, the anti-PD-1 antibody is nivolumab (CAS Registry Number: 946414-94-4). Nivolumab (Bristol-Myers Squibb/Ono), also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. In some embodiments, the anti-PD-1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPGKGLEWVAVIWY
DGSKRYYADSVKGRFTISRDN SKNTLFLQMNSLRAEDTAVYYCATNDDYWGQGLTVTVSS
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQKTYTCNV DHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKGLPSSIEKISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEAL
HNHYTQKSLSLGLGK (SEQ ID NO: 11), and

(b) the light chain comprises the amino acid sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT
GIPARFSGSGGTDFLTITSSLEPEDFAVYYCQQSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSK
ADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 12).

[0119] In some embodiments, the anti-PD-1 antibody comprises the six HVR sequences from SEQ ID NO: 11 and SEQ ID NO: 12 (*e.g.*, the three heavy chain HVRs from SEQ ID NO: 11 and the three light chain HVRs from SEQ ID NO: 12). In some embodiments, the anti-PD-1 antibody

comprises the heavy chain variable domain from SEQ ID NO: 11 and the light chain variable domain from SEQ ID NO: 12.

[0120] In some embodiments, the anti-PD-1 antibody is pembrolizumab (CAS Registry Number: 1374853-91-4). Pembrolizumab (Merck), also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. In some embodiments, the anti-PD-1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

QVQLVQSGVEVKKPGASVKVSCASGYTFTNYYMYWVRQAPGQGLEWMGG
INPSNGGTNFNEKFKNRVTLTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYW
GQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVDPKPSNTKVDKRVERESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK
PREEQFNSTYRVSVLTVHLHQLDNLNGKEYKCKVSNKGLPSSIEKTISKAK
GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCVMHEALHNHYTQKSLSLGLK (SEQ
ID NO: 13), and

(b) the light chain comprises the amino acid sequence:

EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLAAYLES
GVPARFSGSGSGTDFTLTISLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 14).

[0121] In some embodiments, the anti-PD-1 antibody comprises the six HVR sequences from SEQ ID NO: 13 and SEQ ID NO: 14 (*e.g.*, the three heavy chain HVRs from SEQ ID NO: 13 and the three light chain HVRs from SEQ ID NO: 14). In some embodiments, the anti-PD-1 antibody comprises the heavy chain variable domain from SEQ ID NO: 13 and the light chain variable domain from SEQ ID NO: 14.

[0122] In some embodiments, the anti-PD-1 antibody is MEDI-0680 (AMP-514; AstraZeneca). MEDI-0680 is a humanized IgG4 anti-PD-1 antibody.

[0123] In some embodiments, the anti-PD-1 antibody is PDR001 (CAS Registry No. 1859072-53-9; Novartis). PDR001 is a humanized IgG4 anti-PD1 antibody that blocks the binding of PDL1 and PDL2 to PD-1.

[0124] In some embodiments, the anti-PD-1 antibody is REGN2810 (Regeneron). REGN2810 is a human anti-PD1 antibody.

[0125] In some embodiments, the anti-PD-1 antibody is BGB-108 (BeiGene). In some embodiments, the anti-PD-1 antibody is BGB-A317 (BeiGene).

[0126] In some embodiments, the anti-PD-1 antibody is JS-001 (Shanghai Junshi). JS-001 is a humanized anti-PD1 antibody.

[0127] In some embodiments, the anti-PD-1 antibody is STI-A1110 (Sorrento). STI-A1110 is a human anti-PD1 antibody.

[0128] In some embodiments, the anti-PD-1 antibody is INCSHR-1210 (Incyte). INCSHR-1210 is a human IgG4 anti-PD1 antibody.

[0129] In some embodiments, the anti-PD-1 antibody is PF-06801591 (Pfizer).

[0130] In some embodiments, the anti-PD-1 antibody is TSR-042 (also known as ANB011; Tesaro/AnaptysBio).

[0131] In some embodiments, the anti-PD-1 antibody is AM0001 (ARMO Biosciences).

[0132] In some embodiments, the anti-PD-1 antibody is ENUM 244C8 (Enumeral Biomedical Holdings). ENUM 244C8 is an anti-PD1 antibody that inhibits PD-1 function without blocking binding of PDL1 to PD-1.

[0133] In some embodiments, the anti-PD-1 antibody is ENUM 388D4 (Enumeral Biomedical Holdings). ENUM 388D4 is an anti-PD1 antibody that competitively inhibits binding of PDL1 to PD-1.

[0134] In some embodiments, the PD-1 antibody comprises the six HVR sequences (*e.g.*, the three heavy chain HVRs and the three light chain HVRs) and/or the heavy chain variable domain and light chain variable domain from a PD-1 antibody described in WO2015/112800 (Applicant: Regeneron), WO2015/112805 (Applicant: Regeneron), WO2015/112900 (Applicant: Novartis), US20150210769 (Assigned to Novartis), WO2016/089873 (Applicant: Celgene), WO2015/035606 (Applicant: Beigene), WO2015/085847 (Applicants: Shanghai Hengrui Pharmaceutical/Jiangsu Hengrui Medicine), WO2014/206107 (Applicants: Shanghai Junshi Biosciences/Junmeng Biosciences), WO2012/145493 (Applicant: Amplimmune), US9205148 (Assigned to MedImmune), WO2015/119930 (Applicants: Pfizer/Merck), WO2015/119923 (Applicants: Pfizer/Merck), WO2016/032927 (Applicants: Pfizer/Merck), WO2014/179664 (Applicant: AnaptysBio), WO2016/106160 (Applicant: Enumeral), and WO2014/194302 (Applicant: Sorrento).

[0135] In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. AMP-224 (CAS Registry No. 1422184-00-6; GlaxoSmithKline/MedImmune), also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0136] In some embodiments, the PD-1 binding antagonist is a peptide or small molecule compound. In some embodiments, the PD-1 binding antagonist is AUNP-12 (PierreFabre/Aurigene).

See, e.g., WO2012/168944, WO2015/036927, WO2015/044900, WO2015/033303, WO2013/144704, WO2013/132317, and WO2011/161699.

[0137] In some embodiments, the PDL1 binding antagonist is a small molecule that inhibits PD-1. In some embodiments, the PDL1 binding antagonist is a small molecule that inhibits PDL1. In some embodiments, the PDL1 binding antagonist is a small molecule that inhibits PDL1 and VISTA. In some embodiments, the PDL1 binding antagonist is CA-170 (also known as AUPM-170). In some embodiments, the PDL1 binding antagonist is a small molecule that inhibits PDL1 and TIM3. In some embodiments, the small molecule is a compound described in WO2015/033301 and WO2015/033299.

[0138] In some embodiments, the PD-1 axis binding antagonist is an anti-PDL1 antibody. A variety of anti-PDL1 antibodies are contemplated and described herein. In any of the embodiments herein, the isolated anti-PDL1 antibody can bind to a human PDL1, for example a human PDL1 as shown in UniProtKB/Swiss-Prot Accession No.Q9NZQ7.1, or a variant thereof. In some embodiments, the anti-PDL1 antibody is capable of inhibiting binding between PDL1 and PD-1 and/or between PDL1 and B7-1. In some embodiments, the anti-PDL1 antibody is a monoclonal antibody. In some embodiments, the anti-PDL1 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and F(ab')₂ fragments. In some embodiments, the anti-PDL1 antibody is a humanized antibody. In some embodiments, the anti-PDL1 antibody is a human antibody. Examples of anti-PDL1 antibodies useful for the methods of this invention, and methods for making thereof are described in PCT patent application WO 2010/077634 A1 and US Patent No. 8,217,149, which are incorporated herein by reference.

[0139] In some embodiments, the anti-PDL1 antibody comprises a heavy chain variable region and a light chain variable region, wherein:

(a) the heavy chain variable region comprises an HVR-H1, HVR-H2, and HVR-H3 sequence of GFTFSDSWIH (SEQ ID NO: 1), AWISPYGGSTYYADSVKG (SEQ ID NO: 2) and RHWPGGFDY (SEQ ID NO: 3), respectively, and

(b) the light chain variable region comprises an HVR-L1, HVR-L2, and HVR-L3 sequence of RASQDVSTAVA (SEQ ID NO: 4), SASFLYS (SEQ ID NO: 5) and QQYLYHPAT (SEQ ID NO: 6), respectively.

[0140] In some embodiments, the anti-PDL1 antibody is MPDL3280A, also known as atezolizumab and TECENTRIQ® (CAS Registry Number: 1422185-06-5). In some embodiments, the anti-PDL1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain variable region sequence comprises the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO: 7), and

(b) the light chain variable region sequence comprises the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSGSGTDFLTISLQPEDFATYYCQQLYHPATFGQGTKVEIKR (SEQ ID NO: 8).

[0141] In some embodiments, the anti-PDL1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWRQAPGKGLEWVAVISPYGGSTYYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGLTVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
HNHYTQKSLSLSPG (SEQ ID NO:9), and

(b) the light chain comprises the amino acid sequence:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSGSGTDFLTISLQPEDFATYYCQQLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEK
HKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO:10).

[0142] In some embodiments, the anti-PDL1 antibody is avelumab (CAS Registry Number: 1537032-82-8). Avelumab, also known as MSB0010718C, is a human monoclonal IgG1 anti-PDL1 antibody (Merck KGaA, Pfizer). In some embodiments, the anti-PDL1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

EVQLLES GGGLVQPGGSLRLSCAASGFTFSSYIMMWVRQAPGKGLEWVSSIYPSGGITFYAD
TVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIKLGTVTTVDYWGQGLTVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
HNHYTQKSLSLSPG (SEQ ID NO:15), and

(b) the light chain comprises the amino acid sequence:

QSALTQPASVSGSPGQSITISCTGTSSDVGGINYSWYQQHPGKAPKLMYDVSNRPSGVSN
RFSGSKSGNTASLTISGLQAEDADYYCSSYTSSSTRVFGTGTKVTVLGQPKANPTVTLFPPSS

EELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS (SEQ ID NO: 16).

[0143] In some embodiments, the anti-PDL1 antibody comprises the six HVR sequences from SEQ ID NO: 15 and SEQ ID NO: 16 (*e.g.*, the three heavy chain HVRs from SEQ ID NO: 15 and the three light chain HVRs from SEQ ID NO: 16). In some embodiments, the anti-PDL1 antibody comprises the heavy chain variable domain from SEQ ID NO: 15 and the light chain variable domain from SEQ ID NO: 16.

[0144] In some embodiments, the anti-PDL1 antibody is durvalumab (CAS Registry Number: 1428935-60-7). Durvalumab, also known as MEDI4736, is an Fc optimized human monoclonal IgG1 kappa anti-PDL1 antibody (MedImmune, AstraZeneca) described in WO2011/066389 and US2013/034559. In some embodiments, the anti-PDL1 antibody comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCAREGGWFGELAFDYWGQGLTVTS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVDFSCSV MHEALHNHYTQKSLSLSPG (SEQ ID NO: 17), and

(b) the light chain comprises the amino acid sequence:

EIVLTQSPGTLSPGERATLSCRASQRVSSSYLAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGGTDFTLISRLEPEDFAVYYCQQYGSLPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 18).

[0145] In some embodiments, the anti-PDL1 antibody comprises the six HVR sequences from SEQ ID NO: 17 and SEQ ID NO: 18 (*e.g.*, the three heavy chain HVRs from SEQ ID NO: 17 and the three light chain HVRs from SEQ ID NO: 18). In some embodiments, the anti-PDL1 antibody comprises the heavy chain variable domain from SEQ ID NO: 17 and the light chain variable domain from SEQ ID NO: 18.

[0146] In some embodiments, the anti-PDL1 antibody is MDX-1105 (Bristol Myers Squibb). MDX-1105, also known as BMS-936559, is an anti-PDL1 antibody described in WO2007/005874.

[0147] In some embodiments, the anti-PDL1 antibody is LY3300054 (Eli Lilly).

[0148] In some embodiments, the anti-PDL1 antibody is STI-A1014 (Sorrento). STI-A1014 is a human anti-PDL1 antibody.

[0149] In some embodiments, the anti-PDL1 antibody is KN035 (Suzhou Alphamab). KN035 is single-domain antibody (dAB) generated from a camel phage display library.

[0150] In some embodiments, the anti-PDL1 antibody comprises a cleavable moiety or linker that, when cleaved (*e.g.*, by a protease in the tumor microenvironment), activates an antibody antigen binding domain to allow it to bind its antigen, *e.g.*, by removing a non-binding steric moiety. In some embodiments, the anti-PDL1 antibody is CX-072 (CytomX Therapeutics).

[0151] In some embodiments, the PDL1 antibody comprises the six HVR sequences (*e.g.*, the three heavy chain HVRs and the three light chain HVRs) and/or the heavy chain variable domain and light chain variable domain from a PDL1 antibody described in US20160108123 (Assigned to Novartis), WO2016/000619 (Applicant: Beigene), WO2012/145493 (Applicant: Amplimmune), US9205148 (Assigned to MedImmune), WO2013/181634 (Applicant: Sorrento), and WO2016/061142 (Applicant: Novartis).

[0152] In a still further specific aspect, the antibody further comprises a human or murine constant region. In a still further aspect, the human constant region is selected from the group consisting of IgG1, IgG2, IgG2, IgG3, and IgG4. In a still further specific aspect, the human constant region is IgG1. In a still further aspect, the murine constant region is selected from the group consisting of IgG1, IgG2A, IgG2B, and IgG3. In a still further aspect, the murine constant region is IgG2A.

[0153] In a still further specific aspect, the antibody has reduced or minimal effector function. In a still further specific aspect the minimal effector function results from an “effector-less Fc mutation” or aglycosylation mutation. In still a further embodiment, the effector-less Fc mutation is an N297A or D265A/N297A substitution in the constant region. In some embodiments, the isolated anti-PDL1 antibody is aglycosylated. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. Removal of glycosylation sites from an antibody is conveniently accomplished by altering the amino acid sequence such that one of the above-described tripeptide sequences (for N-linked glycosylation sites) is removed. The alteration may be made by substitution of an asparagine, serine or threonine residue within the glycosylation site with another amino acid residue (*e.g.*, glycine, alanine or a conservative substitution).

[0154] In a still further embodiment, the present disclosure provides for compositions comprising any of the above described anti-PDL1 antibodies in combination with at least one pharmaceutically-acceptable carrier.

[0155] In a still further embodiment, the present disclosure provides for a composition comprising an anti-PDL1, an anti-PD-1, or an anti-PDL2 antibody or antigen binding fragment thereof as provided herein and at least one pharmaceutically acceptable carrier. In some embodiments, the anti-PDL1, anti-PD-1, or anti-PDL2 antibody or antigen binding fragment thereof administered to the individual is a composition comprising one or more pharmaceutically acceptable carrier. Any of the pharmaceutically acceptable carriers described herein or known in the art may be used.

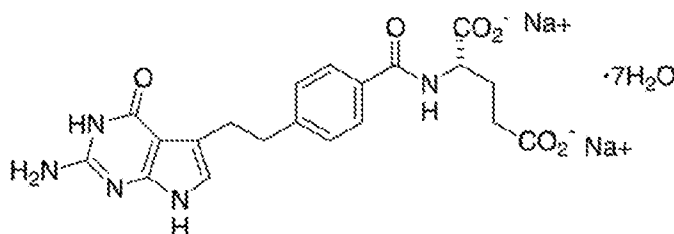
IV. Antimetabolites and Platinum Agents

Antimetabolites

[0156] Antimetabolites (*e.g.*, pemetrexed, 5-fluorouracil, 6-mercaptopurine, capecitabine, cytarabine, floxuridine, fludarabine, hydroxycarbamide, methotrexate, and others) are widely used antitumor drugs that interfere with one or more enzymes necessary for DNA synthesis. Antimetabolites typically act by a variety of mechanisms including, *e.g.*, incorporation into nucleic acids, thereby triggering apoptosis, or, *e.g.*, competition for binding sites of enzymes involved in nucleotide synthesis, thereby depleting the supply required for DNA and/or RNA replication and cell proliferation.

[0157] Pemetrexed is an exemplary antimetabolite used in the methods described herein. Pemetrexed is a folic acid analogue. The drug substance, pemetrexed disodium heptahydrate, has the chemical name L-glutamic acid, N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate with a molecular formula of $C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ and a molecular weight of 597.49.

[0158] Pemetrexed disodium heptahydrate has the following structure:



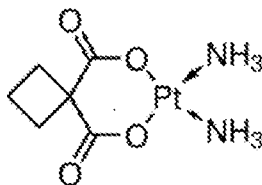
[0159] Pemetrexed inhibits multiple folate-dependent enzymes used in thymine and purine synthesis, namely, thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT) (*see Shih et al. (1997) Cancer Res. 57:1116-23*). By

inhibiting the formation of precursor purine and pyrimidine nucleotides, pemetrexed prevents the formation of DNA and RNA, which are required for the growth and survival of both normal cells and cancer cells. Pemetrexed is commercially available as ALIMTA®, GIOPEM, PEXATE, PEMANAT, PEMEX, PEMMET, PEXATE, RELITREXED, TEMERAN, CIAMBRA, and others.

Platinum Agents

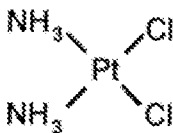
[0160] Platinum agents (such as cisplatin, carboplatin, oxaliplatin, and satraplatin) are widely used antitumor drugs that cause crosslinking of DNA as monoadduct, interstrand crosslinks, intrastrand crosslinks or DNA protein crosslinks. Platinum agents typically act on the adjacent N-7 position of guanine, forming a 1, 2 intrastrand crosslink (Poklar *et al.* (1996). *Proc. Natl. Acad. Sci. U.S.A.* 93 (15): 7606–11; Rudd *et al.* (1995). *Cancer Chemother. Pharmacol.* 35 (4): 323–6). The resultant crosslinking inhibits DNA repair and/or DNA synthesis in cancer cells.

[0161] Carboplatin is an exemplary platinum coordination compound used in the methods described herein. The chemical name for carboplatin is platinum, diammine[1,1-cyclobutanedicarboxylato(2-)- *O,O'*]-, (*SP-4-2*), and carboplatin has the following structural formula:



[0162] Carboplatin is a crystalline powder with the molecular formula of C₆H₁₂N₂O₄Pt and a molecular weight of 371.25. It is soluble in water at a rate of approximately 14 mg/mL, and the pH of a 1% solution is 5 to 7. It is virtually insoluble in ethanol, acetone, and dimethylacetamide. Carboplatin produces predominantly interstrand DNA cross-links, and this effect is cell-cycle nonspecific. Carboplatin is commercially available as PARAPLATIN®, BIOCARB, BLASTOCARB, BLASTOPLATIN, CARBOKEM, CARBOMAX, CARBOPA, CARBOPLAN, CARBOTEEN, CARBOTINAL, CYTOCARB, DUCARB, KARPLAT, KEMOCARB, NAPROPLAT, NEOPLATIN, NISCARBO, ONCOCARBIN, TEVACARB, WOMASTIN, and others.

[0163] Cisplatin is another exemplary platinum coordination compound used in the methods described herein. The chemical name for cisplatin is dichloroplatinum diammoniate, and cisplatin has the following structural formula:



[0164] Cisplatin is an inorganic and water-soluble platinum complex with the molecular formula of $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ and a molecular weight of 300.046. After undergoing hydrolysis, it reacts with DNA to produce both intra and interstrand crosslinks. These crosslinks appear to impair replication and transcription of DNA. The cytotoxicity of cisplatin correlates with cellular arrest in the G2 phase of the cell cycle. Cisplatin is commercially available as PLATINOL®, PLATINOL®-AQ, CDDP, CISPLAN, CISPLAT, PLATIKEM, PLATIONCO, PRACTICIS, PLATICIS, BLASTOLEM, CISMAL, CISPLAN, CISPLATINUM, CISTEEN, DUPLAT, KEMOPLAT, ONCOPLATIN-AQ, PLATINEX, PLATIN, TEVAPLATIN, and others.

V. *Antibody Preparation*

[0165] The antibody described herein is prepared using techniques available in the art for generating antibodies, exemplary methods of which are described in more detail in the following sections.

[0166] The antibody is directed against an antigen of interest (*e.g.*, PD-L1, such as a human PD-L1). Preferably, the antigen is a biologically important polypeptide and administration of the antibody to a mammal suffering from a disorder can result in a therapeutic benefit in that mammal.

[0167] In certain embodiments, an antibody provided herein has a dissociation constant (K_d) of $\leq 1\mu\text{M}$, $\leq 150\text{ nM}$, $\leq 100\text{ nM}$, $\leq 50\text{ nM}$, $\leq 10\text{ nM}$, $\leq 1\text{ nM}$, $\leq 0.1\text{ nM}$, $\leq 0.01\text{ nM}$, or $\leq 0.001\text{ nM}$ (*e.g.*, 10^{-8} M or less, *e.g.*, from 10^{-8} M to 10^{-13} M , *e.g.*, from 10^{-9} M to 10^{-13} M).

[0168] In one embodiment, K_d is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with $5\text{ }\mu\text{g/ml}$ of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate ($\text{pH } 9.6$), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest. The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, $150\text{ }\mu\text{l/well}$ of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations

of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0169] According to another embodiment, K_d is measured using surface plasmon resonance assays using a BIACORE[®]-2000 or a BIACORE[®]-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with *N*-ethyl-*N*'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20[™]) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE[®] Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10⁶ M⁻¹ s⁻¹ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO[™] spectrophotometer (ThermoSpectronic) with a stirred cuvette.

(i) Antigen Preparation

[0170] Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these (e.g., the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source (e.g., cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule. Other antigens and forms thereof useful for preparing antibodies will be apparent to those in the art.

(ii) Exemplary Antibody-Based Methods

[0171] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole

limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

[0172] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

[0173] Monoclonal antibodies of the present disclosure can be made using the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 (1975), and further described, *e.g.*, in Hongo *et al.*, *Hybridoma*, 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981), and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) regarding human-human hybridomas. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 regarding production of monoclonal human natural IgM antibodies from hybridoma cell lines. Human hybridoma technology (Trioma technology) is described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0174] For various other hybridoma techniques, *see, e.g.*, US 2006/258841; US 2006/183887 (fully human antibodies), US 2006/059575; US 2005/287149; US 2005/100546; US 2005/026229; and U.S. Pat. Nos. 7,078,492 and 7,153,507. An exemplary protocol for producing monoclonal antibodies using the hybridoma method is described as follows. In one embodiment, a mouse or other appropriate host animal, such as a hamster, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Antibodies are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of a polypeptide of the present disclosure or a fragment thereof, and an adjuvant, such as monophosphoryl lipid A (MPL)/trehalose dicrynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, Mont.). A polypeptide of the present disclosure (*e.g.*, antigen) or a fragment thereof may be prepared using methods well known in the art, such as recombinant methods, some of which are further described herein. Serum from immunized animals is assayed for anti-antigen antibodies, and booster

immunizations are optionally administered. Lymphocytes from animals producing anti-antigen antibodies are isolated. Alternatively, lymphocytes may be immunized *in vitro*.

[0175] Lymphocytes are then fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. *See, e.g.,* Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986). Myeloma cells may be used that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cells include, but are not limited to, murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0176] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium, *e.g.*, a medium that contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells. Preferably, serum-free hybridoma cell culture methods are used to reduce use of animal-derived serum such as fetal bovine serum, as described, for example, in Even *et al.*, *Trends in Biotechnology*, 24(3), 105-108 (2006).

[0177] Oligopeptides as tools for improving productivity of hybridoma cell cultures are described in Franek, *Trends in Monoclonal Antibody Research*, 111-122 (2005). Specifically, standard culture media are enriched with certain amino acids (alanine, serine, asparagine, proline), or with protein hydrolyzate fractions, and apoptosis may be significantly suppressed by synthetic oligopeptides, constituted of three to six amino acid residues. The peptides are present at millimolar or higher concentrations.

[0178] Culture medium in which hybridoma cells are growing may be assayed for production of monoclonal antibodies that bind to an antibody of the present disclosure. The binding specificity of monoclonal antibodies produced by hybridoma cells may be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). The binding affinity of the monoclonal antibody can be determined, for example, by Scatchard analysis. *See, e.g.,* Munson *et al.*, *Anal. Biochem.* 107:220 (1980).

[0179] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods. *See, e.g.,* Goding, *supra*. Suitable culture media for this purpose include, for

example, D-MEM or RPMI-1640 medium. In addition, hybridoma cells may be grown *in vivo* as ascites tumors in an animal. Monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography. One procedure for isolation of proteins from hybridoma cells is described in US 2005/176122 and U.S. Pat. No. 6,919,436. The method includes using minimal salts, such as lyotropic salts, in the binding process and preferably also using small amounts of organic solvents in the elution process.

(iii) Library-Derived Antibodies

[0180] Antibodies of the present disclosure may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics such as the methods described in Example 3. Additional methods are reviewed, *e.g.*, in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty *et al.*, *Nature* 348:552-554; Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0181] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.*, *EMBO J.*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0182] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

(iv) Chimeric, Humanized and Human Antibodies

[0183] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a “class switched” antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0184] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

[0185] Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann *et al.*, *Nature* 332:323-329 (1988); Queen *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing “resurfacing”); Dall 'Acqua *et al.*, *Methods* 36:43-60 (2005) (describing “FR shuffling”); and Osbourn *et al.*, *Methods* 36:61-68 (2005) and Klimka *et al.*, *Br. J. Cancer*, 83:252-260 (2000) (describing the “guided selection” approach to FR shuffling).

[0186] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (*see, e.g.*, Sims *et al.* *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (*see, e.g.*, Carter *et al.* *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta *et al.* *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (*see, e.g.*, Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (*see, e.g.*, Baca *et al.*, *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok *et al.*, *J. Biol. Chem.* 271:22611-22618 (1996)).

[0187] In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are

described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

[0188] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0189] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al.*, *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0190] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

(v) Antibody Fragments

[0191] Antibody fragments may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance,

and may lead to improved access to solid tumors. For a review of certain antibody fragments, *see* Hudson *et al.* (2003) *Nat. Med.* 9:129-134.

[0192] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.,* Morimoto *et al.*, *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan *et al.*, *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter *et al.*, *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Fab and F(ab')₂ fragment with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Pat. No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). *See* WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. Fv and scFv are the only species with intact combining sites that are devoid of constant regions; thus, they may be suitable for reduced nonspecific binding during *in vivo* use. scFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an scFv. *See* *Antibody Engineering*, ed. Borrebaeck, *supra*. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Pat. No. 5,641,870, for example. Such linear antibodies may be monospecific or bispecific.

(vi) Multispecific Antibodies

[0193] Multispecific antibodies have binding specificities for at least two different epitopes, where the epitopes are usually from different antigens. While such molecules normally will only bind two different epitopes (*i.e.* bispecific antibodies, BsAbs), antibodies with additional specificities such as trispecific antibodies are encompassed by this expression when used herein. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')₂ bispecific antibodies).

[0194] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

[0195] One approach known in the art for making bispecific antibodies is the “knobs-into-holes” or “protuberance-into-cavity” approach (*see, e.g.*, US Pat. No. 5,731,168). In this approach, two immunoglobulin polypeptides (*e.g.*, heavy chain polypeptides) each comprise an interface. An interface of one immunoglobulin polypeptide interacts with a corresponding interface on the other immunoglobulin polypeptide, thereby allowing the two immunoglobulin polypeptides to associate. These interfaces may be engineered such that a “knob” or “protuberance” (these terms may be used interchangeably herein) located in the interface of one immunoglobulin polypeptide corresponds with a “hole” or “cavity” (these terms may be used interchangeably herein) located in the interface of the other immunoglobulin polypeptide. In some embodiments, the hole is of identical or similar size to the knob and suitably positioned such that when the two interfaces interact, the knob of one interface is positionable in the corresponding hole of the other interface. Without wishing to be bound to theory, this is thought to stabilize the heteromultimer and favor formation of the heteromultimer over other species, for example homomultimers. In some embodiments, this approach may be used to promote the heteromultimerization of two different immunoglobulin polypeptides, creating a bispecific antibody comprising two immunoglobulin polypeptides with binding specificities for different epitopes.

[0196] In some embodiments, a knob may be constructed by replacing a small amino acid side chain with a larger side chain. In some embodiments, a hole may be constructed by replacing a large amino acid side chain with a smaller side chain. Knobs or holes may exist in the original interface, or they may be introduced synthetically. For example, knobs or holes may be introduced synthetically by altering the nucleic acid sequence encoding the interface to replace at least one “original” amino acid residue with at least one “import” amino acid residue. Methods for altering nucleic acid sequences may include standard molecular biology techniques well known in the art. The side chain volumes of various amino acid residues are shown in *Table 1* below. In some embodiments, original residues have a small side chain volume (*e.g.*, alanine, asparagine, aspartic acid, glycine, serine, threonine, or valine), and import residues for forming a knob are naturally occurring amino acids and may include arginine, phenylalanine, tyrosine, and tryptophan. In some embodiments, original residues have a large side chain volume (*e.g.*, arginine, phenylalanine, tyrosine, and tryptophan), and import residues for forming a hole are naturally occurring amino acids and may include alanine, serine, threonine, and valine.

Table 1. Properties of Amino Acid Residues

Amino acid	One-letter abbreviation	Mass ^a (daltons)	Volume ^b (Å ³)	Accessible surface area ^c (Å ²)
Alanine (Ala)	A	71.08	88.6	115
Arginine (Arg)	R	156.20	173.4	225

Amino acid	One-letter abbreviation	Mass ^a (daltons)	Volume ^b (Å ³)	Accessible surface area ^c (Å ²)
Asparagine (Asn)	N	114.11	117.7	160
Aspartic Acid (Asp)	D	115.09	111.1	150
Cysteine (Cys)	C	103.14	108.5	135
Glutamine (Gln)	Q	128.14	143.9	180
Glutamic Acid (Glu)	E	129.12	138.4	190
Glycine (Gly)	G	57.06	60.1	75
Histidine (His)	H	137.15	153.2	195
Isoleucine (Ile)	I	113.17	166.7	175
Leucine (Leu)	L	113.17	166.7	170
Lysine (Lys)	K	128.18	168.6	200
Methionine (Met)	M	131.21	162.9	185
Phenylalanine (Phe)	F	147.18	189.9	210
Proline (Pro)	P	97.12	122.7	145
Serine (Ser)	S	87.08	89.0	115
Threonine (Thr)	T	101.11	116.1	140
Tryptophan (Trp)	W	186.21	227.8	255
Tyrosine (Tyr)	Y	163.18	193.6	230
Valine (Val)	V	99.14	140.0	155

^a Molecular weight of amino acid minus that of water. Values from Handbook of Chemistry and Physics, 43rd ed. Cleveland, Chemical Rubber Publishing Co., 1961.

^b Values from A.A. Zamyatin, Prog. Biophys. Mol. Biol. 24:107-123, 1972.

^c Values from C. Chothia, J. Mol. Biol. 105:1-14, 1975. The accessible surface area is defined in Figures 6-20 of this reference.

[0197] In some embodiments, original residues for forming a knob or hole are identified based on the three-dimensional structure of the heteromultimer. Techniques known in the art for obtaining a three-dimensional structure may include X-ray crystallography and NMR. In some embodiments, the interface is the CH3 domain of an immunoglobulin constant domain. In these embodiments, the CH3/CH3 interface of human IgG₁ involves sixteen residues on each domain located on four anti-parallel β -strands. Without wishing to be bound to theory, mutated residues are preferably located on the two central anti-parallel β -strands to minimize the risk that knobs can be accommodated by the surrounding solvent, rather than the compensatory holes in the partner CH3 domain. In some embodiments, the mutations forming corresponding knobs and holes in two immunoglobulin polypeptides correspond to one or more pairs provided in *Table 2*.

*Table 2. Exemplary sets of corresponding knob-and hole-forming mutations**

CH3 of first immunoglobulin	CH3 of second immunoglobulin
T366Y	Y407T
T366W	Y407A
F405A	T394W
Y407T	T366Y
T366Y:F405A	T394W:Y407T
T366W:F405W	T394S:Y407A
F405W:Y407A	T366W:T394S
F405W	T394S

* Mutations are denoted by the original residue, followed by the position using the Kabat numbering system, and then the import residue (all residues are given in single-letter amino acid code). Multiple mutations are separated by a colon.

[0198] In some embodiments, an immunoglobulin polypeptide comprises a CH3 domain comprising one or more amino acid substitutions listed in *Table 2* above. In some embodiments, a bispecific antibody comprises a first immunoglobulin polypeptide comprising a CH3 domain comprising one or more amino acid substitutions listed in the left column of *Table 2*, and a second immunoglobulin polypeptide comprising a CH3 domain comprising one or more corresponding amino acid substitutions listed in the right column of *Table 2*.

[0199] Following mutation of the DNA as discussed above, polynucleotides encoding modified immunoglobulin polypeptides with one or more corresponding knob- or hole-forming mutations may be expressed and purified using standard recombinant techniques and cell systems known in the art. See, e.g., U.S. Pat. Nos. 5,731,168; 5,807,706; 5,821,333; 7,642,228; 7,695,936; 8,216,805; U.S. Pub. No. 2013/0089553; and Spiess *et al.*, Nature Biotechnology 31: 753-758, 2013. Modified immunoglobulin polypeptides may be produced using prokaryotic host cells, such as *E. coli*, or eukaryotic host cells, such as CHO cells. Corresponding knob- and hole-bearing immunoglobulin polypeptides may be expressed in host cells in co-culture and purified together as a heteromultimer, or they may be expressed in single cultures, separately purified, and assembled *in vitro*. In some embodiments, two strains of bacterial host cells (one expressing an immunoglobulin polypeptide with a knob, and the other expressing an immunoglobulin polypeptide with a hole) are co-cultured using standard bacterial culturing techniques known in the art. In some embodiments, the two strains may be mixed in a specific ratio, e.g., so as to achieve equal expression levels in culture. In some embodiments, the two strains may be mixed in a 50:50, 60:40, or 70:30 ratio. After polypeptide expression, the cells may be lysed together, and protein may be extracted. Standard techniques known in the art that allow for measuring the abundance of homo-multimeric vs. hetero-multimeric species may include size exclusion chromatography. In some embodiments, each modified immunoglobulin polypeptide is expressed separately using standard recombinant techniques, and they may be assembled together *in vitro*. Assembly may be achieved, for example, by purifying each

modified immunoglobulin polypeptide, mixing and incubating them together in equal mass, reducing disulfides (*e.g.*, by treating with dithiothreitol), concentrating, and reoxidizing the polypeptides.

Formed bispecific antibodies may be purified using standard techniques including cation-exchange chromatography and measured using standard techniques including size exclusion chromatography.

For a more detailed description of these methods, *see* Speiss *et al.*, *Nat Biotechnol* 31:753-8, 2013. In some embodiments, modified immunoglobulin polypeptides may be expressed separately in CHO cells and assembled *in vitro* using the methods described above.

[0200] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is typical to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0201] In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies *see*, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

[0202] According to another approach described in WO96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. One interface comprises at least a part of the C_H 3 domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains

with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0203] Bispecific antibodies include cross-linked or “heteroconjugate” antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Pat. No. 4,676,980, along with a number of cross-linking techniques.

[0204] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0205] Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody.

[0206] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The “diabody” technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming

two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al, *J. Immunol.*, 152:5368 (1994).

[0207] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. *Tuft et al. J. Immunol.* 147: 60 (1991).

(vii) Single-Domain Antibodies

[0208] In some embodiments, an antibody of the present disclosure is a single-domain antibody. A single-domain antibody is a single polypeptide chain comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; *see, e.g.*, U.S. Pat. No. 6,248,516 B1). In one embodiment, a single-domain antibody consists of all or a portion of the heavy chain variable domain of an antibody.

(viii) Antibody Variants

[0209] In some embodiments, amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibody may be prepared by introducing appropriate changes into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid alterations may be introduced in the subject antibody amino acid sequence at the time that sequence is made.

(ix) Substitution, Insertion, and Deletion Variants

[0210] In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in *Table 3*. More substantial changes are provided in Table 1 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

Table 3. Conservative Substitutions.

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln

Original Residue	Exemplary Substitutions	Preferred Substitutions
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0211] Amino acids may be grouped according to common side-chain properties:

- a.** hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
- b.** neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- c.** acidic: Asp, Glu;
- d.** basic: His, Lys, Arg;
- e.** residues that influence chain orientation: Gly, Pro;
- f.** aromatic: Trp, Tyr, Phe.

[0212] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0213] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one

or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.*, binding affinity).

[0214] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR “hotspots,” *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom *et al.* in *Methods in Molecular Biology* 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0215] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR “hotspots” or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0216] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0217] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as

intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

(x) Glycosylation variants

[0218] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0219] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. *See, e.g.*, Wright *et al.* *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the present disclosure may be made in order to create antibody variants with certain improved properties.

[0220] In one embodiment, antibody variants are provided comprising an Fc region wherein a carbohydrate structure attached to the Fc region has reduced fucose or lacks fucose, which may improve ADCC function. Specifically, antibodies are contemplated herein that have reduced fucose relative to the amount of fucose on the same antibody produced in a wild-type CHO cell. That is, they are characterized by having a lower amount of fucose than they would otherwise have if produced by native CHO cells (*e.g.*, a CHO cell that produce a native glycosylation pattern, such as, a CHO cell containing a native FUT8 gene). In certain embodiments, the antibody is one wherein less than about 50%, 40%, 30%, 20%, 10%, or 5% of the N-linked glycans thereon comprise fucose. For example, the amount of fucose in such an antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. In certain embodiments, the antibody is one wherein none of the N-linked glycans thereon comprise fucose, *i.e.*, wherein the antibody is completely without fucose, or has no fucose or is afucosylated. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (*e.g.*, complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. *See, e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US

2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al. J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al. Arch. Biochem. Biophys.* 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8*, knockout CHO cells (*see, e.g.*, Yamane-Ohnuki *et al. Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. *et al., Biotechnol. Bioeng.* 94(4):680-688 (2006); and WO2003/085107).

[0221] Antibody variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); US 2005/0123546 (Umana *et al.*), and Ferrara *et al.*, *Biotechnology and Bioengineering*, 93(5): 851-861 (2006). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0222] In certain embodiments, the antibody variants comprising an Fc region described herein are capable of binding to an FcγRIII. In certain embodiments, the antibody variants comprising an Fc region described herein have ADCC activity in the presence of human effector cells or have increased ADCC activity in the presence of human effector cells compared to the otherwise same antibody comprising a human wild-type IgG1Fc region.

(xi) Fc region variants

[0223] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.*, a substitution) at one or more amino acid positions.

[0224] In certain embodiments, the present disclosure contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays

can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc γ R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc(RIII only), whereas monocytes express Fc(RI, Fc(RII and Fc(RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (*see, e.g.* Hellstrom, I. *et al. Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I *et al., Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (*see* Bruggemann, M. *et al., J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (*see, for example*, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.,* in an animal model such as that disclosed in Clynes *et al. Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. *See, e.g.,* C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (*see, for example*, Gazzano-Santoro *et al., J. Immunol. Methods* 202:163 (1996); Cragg, M.S. *et al., Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (*see, e.g.,* Petkova, S.B. *et al. Int'l. Immunol.* 18(12):1759-1769 (2006)).

[0225] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called “DANA” Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0226] Certain antibody variants with improved or diminished binding to FcRs are described. (*See, e.g.,* U.S. Patent No. 6,737,056; WO 2004/056312, and Shields *et al., J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0227] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.,* substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues). In an exemplary embodiment, the antibody comprising the following amino acid substitutions in its Fc region: S298A, E333A, and K334A.

[0228] In some embodiments, alterations are made in the Fc region that result in altered (*i.e.,* either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.,*

as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie *et al.* *J. Immunol.* 164: 4178-4184 (2000).

[0229] Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, *J. Immunol.* 117:587 (1976) and Kim *et al.*, *J. Immunol.* 24:249 (1994)), are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

VII. *Pharmaceutical Compositions and Formulations*

[0230] Also provided herein are pharmaceutical compositions and formulations, *e.g.*, for the treatment of lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) comprising a PD-1 axis binding antagonist (such as atezolizumab), a platinum agent (such as carboplatin or cisplatin), and an antimetabolite (such as pemetrexed). In some embodiments, the pharmaceutical compositions and formulations further comprise a pharmaceutically acceptable carrier.

[0231] In some embodiments, an anti-PDL1 antibody described herein (such as atezolizumab) is in a formulation comprising the antibody at an amount of about 60 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose in a concentration of about 120 mM, and polysorbate (*e.g.*, polysorbate 20) in a concentration of 0.04% (w/v), and the formulation has a pH of about 5.8. In some embodiments, the anti-PDL1 antibody described herein (such as atezolizumab) is in a formulation comprising the antibody in an amount of about 125 mg/mL, histidine acetate in a concentration of about 20 mM, sucrose is in a concentration of about 240 mM, and polysorbate (*e.g.*, polysorbate 20) in a concentration of 0.02% (w/v), and the formulation has a pH of about 5.5.

[0232] After preparation of the antibody of interest (*e.g.*, techniques for producing antibodies which can be formulated as disclosed herein are elaborated herein and are known in the art), the pharmaceutical formulation comprising it is prepared. In certain embodiments, the antibody to be formulated has not been subjected to prior lyophilization and the formulation of interest herein is an aqueous formulation. In certain embodiments, the antibody is a full length antibody. In one embodiment, the antibody in the formulation is an antibody fragment, such as an F(ab')₂, in which case problems that may not occur for the full length antibody (such as clipping of the antibody to Fab) may need to be addressed. The therapeutically effective amount of antibody present in the formulation is determined by taking into account the desired dose volumes and mode(s) of administration, for example. From about 25 mg/mL to about 150 mg/mL, or from about 30 mg/mL to

about 140 mg/mL, or from about 35 mg/mL to about 130 mg/mL, or from about 40 mg/mL to about 120 mg/mL, or from about 50 mg/mL to about 130 mg/mL, or from about 50 mg/mL to about 125 mg/mL, or from about 50 mg/mL to about 120 mg/mL, or from about 50 mg/mL to about 110 mg/mL, or from about 50 mg/mL to about 100 mg/mL, or from about 50 mg/mL to about 90 mg/mL, or from about 50 mg/mL to about 80 mg/mL, or from about 54 mg/mL to about 66 mg/mL is an exemplary antibody concentration in the formulation.

[0233] An aqueous formulation is prepared comprising the antibody in a pH-buffered solution. In some embodiments, the buffer of the present disclosure has a pH in the range from about 5.0 to about 7.0. In certain embodiments the pH is in the range from about 5.0 to about 6.5, the pH is in the range from about 5.0 to about 6.4, in the range from about 5.0 to about 6.3, the pH is in the range from about 5.0 to about 6.2, the pH is in the range from about 5.0 to about 6.1, the pH is in the range from about 5.5 to about 6.1, the pH is in the range from about 5.0 to about 6.0, the pH is in the range from about 5.0 to about 5.9, the pH is in the range from about 5.0 to about 5.8, the pH is in the range from about 5.1 to about 6.0, the pH is in the range from about 5.2 to about 6.0, the pH is in the range from about 5.3 to about 6.0, the pH is in the range from about 5.4 to about 6.0, the pH is in the range from about 5.5 to about 6.0, the pH is in the range from about 5.6 to about 6.0, the pH is in the range from about 5.7 to about 6.0, or the pH is in the range from about 5.8 to about 6.0. In some embodiments, the formulation has a pH of 6.0 or about 6.0. In some embodiments, the formulation has a pH of 5.9 or about 5.9. In some embodiments, the formulation has a pH of 5.8 or about 5.8. In some embodiments, the formulation has a pH of 5.7 or about 5.7. In some embodiments, the formulation has a pH of 5.6 or about 5.6. In some embodiments, the formulation has a pH of 5.5 or about 5.5. In some embodiments, the formulation has a pH of 5.4 or about 5.4. In some embodiments, the formulation has a pH of 5.3 or about 5.3. In some embodiments, the formulation has a pH of 5.2 or about 5.2. Examples of buffers that will control the pH within this range include histidine (such as L-histidine) or sodium acetate. In certain embodiments, the buffer contains histidine acetate or sodium acetate in the concentration of about 15 mM to about 25 mM. In some embodiments, the buffer contains histidine acetate or sodium acetate in the concentration of about 15 mM to about 25 mM, about 16 mM to about 25 mM, about 17 mM to about 25 mM, about 18 mM to about 25 mM, about 19 mM to about 25 mM, about 20 mM to about 25 mM, about 21 mM to about 25 mM, about 22 mM to about 25 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, or about 25 mM. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.0. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.1. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.2. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.3. In one embodiment, the buffer is histidine acetate or sodium

acetate in an amount of about 20 mM, pH 5.4. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.5. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.6. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.7. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.8. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 5.9. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 6.0. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 6.1. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 6.2. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 20 mM, pH 6.3. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.2. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.3. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.4. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.5. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.6. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.7. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.8. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 5.9. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 6.0. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 6.1. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 6.2. In one embodiment, the buffer is histidine acetate or sodium acetate in an amount of about 25 mM, pH 6.3.

[0234] In some embodiments, the formulation further comprises sucrose in an amount of about 60 mM to about 240 mM. In some embodiments, sucrose in the formulation is about 60 mM to about 230 mM, about 60 mM to about 220 mM, about 60 mM to about 210 mM, about 60 mM to about 200 mM, about 60 mM to about 190 mM, about 60 mM to about 180 mM, about 60 mM to about 170 mM, about 60 mM to about 160 mM, about 60 mM to about 150 mM, about 60 mM to about 140 mM, about 80 mM to about 240 mM, about 90 mM to about 240 mM, about 100 mM to about 240 mM, about 110 mM to about 240 mM, about 120 mM to about 240 mM, about 130 mM to about 240 mM, about 140 mM to about 240 mM, about 150 mM to about 240 mM, about 160 mM to about 240 mM, about 170 mM to about 240 mM, about 180 mM to about 240 mM, about 190 mM to about 240 mM, about 200 mM to about 240 mM, about 80 mM to about 160 mM, about 100 mM to about 140 mM, or about 110 mM to about 130 mM. In some embodiments, sucrose in the formulation is about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM,

about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, or about 240 mM.

[0235] In some embodiments, the antibody concentration in the formulation is about 40 mg/ml to about 125 mg/ml. In some embodiments, the antibody concentration in the formulation is about 40 mg/ml to about 120 mg/ml, about 40 mg/ml to about 110 mg/ml, about 40 mg/ml to about 100 mg/ml, about 40 mg/ml to about 90 mg/ml, about 40 mg/ml to about 80 mg/ml, about 40 mg/ml to about 70 mg/ml, about 50 mg/ml to about 120 mg/ml, about 60 mg/ml to about 120 mg/ml, about 70 mg/ml to about 120 mg/ml, about 80 mg/ml to about 120 mg/ml, about 90 mg/ml to about 120 mg/ml, or about 100 mg/ml to about 120 mg/ml. In some embodiments, the antibody concentration in the formulation is about 60 mg/ml. In some embodiments, the antibody concentration in the formulation is about 65 mg/ml. In some embodiments, the antibody concentration in the formulation is about 70 mg/ml. In some embodiments, the antibody concentration in the formulation is about 75 mg/ml. In some embodiments, the antibody concentration in the formulation is about 80 mg/ml. In some embodiments, the antibody concentration in the formulation is about 85 mg/ml. In some embodiments, the antibody concentration in the formulation is about 90 mg/ml. In some embodiments, the antibody concentration in the formulation is about 95 mg/ml. In some embodiments, the antibody concentration in the formulation is about 100 mg/ml. In some embodiments, the antibody concentration in the formulation is about 110 mg/ml. In some embodiments, the antibody concentration in the formulation is about 125 mg/ml.

[0236] In some embodiments, a surfactant is added to the antibody formulation. Exemplary surfactants include nonionic surfactants such as polysorbates (*e.g.*, polysorbates 20, 80 etc.) or poloxamers (*e.g.*, poloxamer 188, etc.). The amount of surfactant added is such that it reduces aggregation of the formulated antibody and/or minimizes the formation of particulates in the formulation and/or reduces adsorption. For example, the surfactant may be present in the formulation in an amount from about 0.001% to about 0.5% (w/v). In some embodiments, the surfactant (*e.g.*, polysorbate 20) is from about 0.005% to about 0.2%, from about 0.005% to about 0.1%, from about 0.005% to about 0.09%, from about 0.005% to about 0.08%, from about 0.005% to about 0.07%, from about 0.005% to about 0.06%, from about 0.005% to about 0.05%, from about 0.005% to about 0.04%, from about 0.008% to about 0.06%, from about 0.01% to about 0.06%, from about 0.02% to about 0.06%, from about 0.01% to about 0.05%, or from about 0.02% to about 0.04%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.005% or about 0.005%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.006% or about 0.006%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.007% or about 0.007%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.008% or about 0.008%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in

the formulation in an amount of 0.009% or about 0.009%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.01% or about 0.01%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.02% or about 0.02%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.03% or about 0.03%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.04% or about 0.04%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.05% or about 0.05%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.06% or about 0.06%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.07% or about 0.07%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.08% or about 0.08%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.1% or about 0.1%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.2% or about 0.2%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.3% or about 0.3%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.4% or about 0.4%. In certain embodiments, the surfactant (*e.g.*, polysorbate 20) is present in the formulation in an amount of 0.5% or about 0.5%.

[0237] In one embodiment, the formulation contains the above-identified agents (*e.g.*, antibody, buffer, sucrose, and/or surfactant) and is essentially free of one or more preservatives, such as benzyl alcohol, phenol, m-cresol, chlorobutanol and benzethonium Cl. In another embodiment, a preservative may be included in the formulation, particularly where the formulation is a multidose formulation. The concentration of preservative may be in the range from about 0.1% to about 2%, preferably from about 0.5% to about 1%. One or more other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the formulation provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; co-solvents; anti-oxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (*e.g.*, Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions. Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968.

In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0238] The formulation herein may also contain more than one protein as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other protein. For example, where the antibody is anti-PDL1 (such as atezolizumab), it may be combined with another agent (*e.g.*, a chemotherapeutic agent, and anti-neoplastic agent).

[0239] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as an antibody or a polypeptide) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0240] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0241] The composition and formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0242] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0243] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

[0244] Pharmaceutical formulations of carboplatin, cisplatin and/or pemetrexed are commercially available. For example, carboplatin is known under a variety of trade names (as described elsewhere herein) including PARAPLATIN®. Cisplatin is known under a variety of trade names (as described elsewhere herein) including PLATINOL®. Pemetrexed is known under a variety of trade names (as described elsewhere herein), including ALIMTA®, GIOPEM, PEXATE, and CIAMBRA. In some embodiments, the carboplatin and/or the pemetrexed are provided in separate containers. In some embodiments, the cisplatin and/or the pemetrexed are provided in separate containers. In some embodiments, the carboplatin and/or the pemetrexed are each used and/or prepared for administration to an individual as described in the prescribing information available with the commercially available product. In some embodiments, the cisplatin and/or the pemetrexed are each used and/or prepared for administration to an individual as described in the prescribing information available with the commercially available product.

VIII. Methods of Treatment

[0245] Provided herein are methods for treating or delaying progression of cancer (such as lung cancer, *e.g.*, non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody), an antimetabolite (*e.g.*, pemetrexed), and a platinum agent (*e.g.*, carboplatin or cisplatin). In some embodiments, the treatment results in a sustained response in the individual after cessation of the treatment. In some embodiments, the treatment extends the progression free survival (PFS) of the individual. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the administering extends the overall survival (OS) of the individual. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5,

15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months.

[0246] The methods described herein may find use in treating conditions where enhanced immunogenicity is desired such as increasing tumor immunogenicity for the treatment of cancer. Also provided herein are methods of enhancing immune function in an individual having (such as lung cancer, *e.g.*, non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) in an individual comprising administering to the individual an effective amount of a PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody), an antimetabolite (*e.g.*, pemetrexed), and a platinum agent (*e.g.*, carboplatin or cisplatin).

[0247] In some embodiments, the lung cancer is non-small cell lung cancer (NSCLC). In some embodiments, the NSCLC is Stage IV NSCLC. In some embodiments, the Stage IV NSCLC is non-squamous NSCLC. In some embodiments, the NSCLC is histologically or cytologically confirmed Stage IV non-squamous NSCLC, according to or as defined by the Union Internationale contre le Cancer/American Joint Committee on Cancer staging system, 7th edition (*see*, *e.g.*, Detterbeck *et al.* (2009) *Chest* 136: 260–71). In some embodiments, the Stage IV NSCLC is of mixed non-small cell histology (*e.g.*, squamous and non-squamous), and the major histological component is or appears to be non-squamous. In some embodiments, the NSCLC is classified as Stage IV if the tumor has grown into nearby structures. In some embodiments, the NSCLC is classified as Stage IV if the tumor has grown into nearby structures and/or has reached proximal lymph nodes. In some embodiments, the NSCLC is classified as Stage IV if the cancer has spread to the other lung from the originally affected lung. In some embodiments, the NSCLC is classified as Stage IV if cancer cells are found in the fluid around the lung (*i.e.*, malignant pleural effusion). In some embodiments, the NSCLC is classified as Stage IV if cancer cells are found in the fluid around the heart (*i.e.*, malignant pericardial infusion). In some embodiments, the NSCLC is classified as Stage IV if the cancer has spread as a single tumor outside the chest, such as to distant lymph node(s), the liver, bones, and/or the brain. In some embodiments, the NSCLC is classified as Stage IV if the cancer has spread as more than one tumor outside the chest, such as to distant lymph node(s), the liver, bones, and/or the brain. In some embodiments, the Stage IV NSCLC is difficult to treat. Further details regarding NSCLC staging are described in American Joint Committee on Cancer. Lung. In: *AJCC Cancer Staging Manual*. 8th ed. New York, NY: Springer; 2017: 431-456.

[0248] In some embodiments, the individual has a poor prognosis. In some embodiments, the individual is a treatment-naïve individual. In some embodiments, a treatment-naïve individual is an individual who has not received prior treatment, *e.g.*, for cancer, for NSCLC, or for Stage IV non-squamous NSCLC. In some embodiments, the treatment naïve individual is an individual who has not received prior treatment for Stage IV non-squamous NSCLC. In some embodiments, the individual is

chemotherapy naïve, *e.g.*, an individual who has not received prior chemotherapy for the treatment of, *e.g.*, cancer, NSCLC, and/or Stage IV non-squamous NSCLC. In some embodiments, the individual has not received treatment for Stage IV non-squamous NSCLC. In some embodiments, the individual has not received prior systemic treatment for Stage IV non-squamous NSCLC.

[0249] In some embodiments, the individual is Asian. In some embodiments, the individual is of Asian descent. In some embodiments the individual is at least 65 years old. In some embodiments, the individual is a never smoker. In some embodiments, a never smoker is an adult who has never smoked, or who has smoked less than 100 cigarettes in his or her lifetime. In some embodiments, the individual does not have liver metastases.

[0250] In some embodiments, the individual is “PD-L1 high.” In some embodiments, a patient is “PD-L1 high” if tumor cells expressing PD-L1 in a pre-treatment sample from the patient total $\geq 50\%$ of the total tumor cells in the sample. In some embodiments, PD-L1 expression on $\geq 50\%$ of the tumor cells in a pretreatment sample is defined / scored as “TC3.” In some embodiments a patient is “PD-L1 high” if tumor-infiltrating immune cells expressing PD-L1 in a pre-treatment sample from the patient total $\geq 10\%$ of the total tumor-filtrating immune cells in the sample. In some embodiments, PD-L1 expression on $\geq 10\%$ of the tumor-infiltrating immune cells in a pretreatment sample is defined / scored as “IC3.” In some embodiments, the pre-treatment sample is a fresh tumor sample. In some embodiments, the pre-treatment sample is a formalin-fixed paraffin-embedded (FFPE) tumor sample. In some embodiments, PD-L1 expression level on the tumor cells and/or the tumor-infiltrating immune cells in the pre-treatment sample is determined via immunohistochemical assay. In some embodiments, the immunohistochemical assay is the VENTANA SP142 assay.

[0251] In some embodiments, a patient is “PD-L1 low” if tumor cells expressing PD-L1 in a pre-treatment sample from the patient total 1% to $<5\%$ of the total tumor cells in the sample. In some embodiments, PD-L1 expression on 1% to $<5\%$ of the tumor cells in a pretreatment sample is defined / scored as “TC1.” In some embodiments, a patient is “PD-L1 low” if tumor cells expressing PD-L1 in a pre-treatment sample from the patient total 5% to $<50\%$ of the total tumor cells in the sample. In some embodiments, PD-L1 expression on 5% to $<50\%$ of the tumor cells in a pretreatment sample is defined / scored as “TC2.” In some embodiments a patient is “PD-L1 low” if tumor-infiltrating immune cells expressing PD-L1 in a pre-treatment sample from the patient total 1% to $<5\%$ of the total tumor-filtrating immune cells in the sample. In some embodiments, PD-L1 expression on 1% to $<5\%$ of the tumor-infiltrating immune cells in a pretreatment sample is defined / scored as “IC1.” In some embodiments a patient is “PD-L1 low” if tumor-infiltrating immune cells expressing PD-L1 in a pre-treatment sample from the patient total 5% to $<10\%$ of the total tumor-filtrating immune cells in the sample. In some embodiments, PD-L1 expression on 5% to $<10\%$ of the tumor-infiltrating immune cells in a pretreatment sample is defined / scored as “IC2.” In some embodiments, the pre-treatment sample is a fresh tumor sample. In some embodiments, the pre-treatment sample is a

formalin-fixed paraffin-embedded (FFPE) tumor sample. In some embodiments, PD-L1 expression level on the tumor cells and/or the tumor-infiltrating immune cells in the pre-treatment sample is determined via immunohistochemical assay. In some embodiments, the immunohistochemical assay is the VENTANA SP142 assay.

[0252] In some embodiments, the individual is “PD-L1 negative.” In some embodiments, a patient is “PD-L1 negative” if tumor cells expressing PD-L1 in a pre-treatment sample from the patient total <1% of the total tumor cells in the sample. In some embodiments, PD-L1 expression on <1% of the tumor cells in a pretreatment sample is defined as “TC0.” In some embodiments a patient is “PD-L1 negative” if tumor-infiltrating immune cells expressing PD-L1 in a pre-treatment sample from the patient total <1% of the total tumor-infiltrating immune cells in the sample. In some embodiments, PD-L1 expression on <1% of the tumor-infiltrating immune cells in a pretreatment sample is defined as “IC0.” In some embodiments, the pre-treatment sample is a fresh tumor sample. In some embodiments, the pre-treatment sample is a formalin-fixed paraffin-embedded (FFPE) tumor sample. In some embodiments, PD-L1 expression level in the tumor cells and/or the tumor-infiltrating immune cells in the pre-treatment sample is determined via immunohistochemical assay. In some embodiments, the immunohistochemical assay is the VENTANA SP142 assay, which is described in further detail elsewhere herein.

[0253] In some embodiments, TC0, TC1, TC2, TC3, IC0, IC1, IC2, and IC3 are defined / scored as summarized in the table below:

Exemplary tumor cell (TC) and tumor-infiltrating immune cell (IC) scoring definitions*

PD-L1 TC staining criteria*		PD-L1 IC staining criteria*	
TC Score	Definition	IC Score	Definition
TC0	< 1%	IC0	< 1%
TC1	≥ 1% and < 5%	IC1	≥ 1% and < 5%
TC2	≥ 5% and < 50%	IC2	≥ 5% and < 10%
TC3	≥ 50%	IC3	≥ 10%

*TC are scored first followed by IC in a stepwise approach

*See the Assessment Guide for the VENTANA PD-L1 (SP142) IHC Assay: Staining of Non-Small Cell Lung Cancer Universal Training. (See [www \(dot\) rocheplus \(dot\) es/content/dam/hcp-portals/spain/documents/formaci%C3%B3n/uropath/PD-L1%20SP142%20Assessment%20v2.5 \(dot\) pdf](http://www.rocheplus.es/content/dam/hcp-portals/spain/documents/formaci%C3%B3n/uropath/PD-L1%20SP142%20Assessment%20v2.5.pdf))

[0254] In some embodiments, the individual has histologically or cytologically confirmed, Stage IV non-squamous NSCLC (per the criteria outlined by the Union Internationale contre le Cancer/American Joint Committee on Cancer staging system, 7th edition, as described in Detterbeck *et al.* (2009) “The new lung cancer staging system.” *Chest*. 136: 260–71). In some embodiments, the individual has NSCLC of mixed non-small cell histology (*i.e.*, squamous and non-squamous), and is considered to have non-squamous NSCLC if the major histological component is or appears to be non-squamous. In some embodiments, the individual does not have a sensitizing mutation in the

EGFR gene. In some embodiments, the individual does not have an *ALK* fusion oncogene. In some embodiments, the individual's *EGFR* and *ALK* status is screened prior to treatment.

[0255] In some embodiments the individual has received prior neo-adjuvant chemotherapy, adjuvant chemotherapy, or chemoradiotherapy with curative intent for non-metastatic disease, and has experienced a treatment-free interval of at least 6 months since the last dose chemotherapy and/or radiotherapy prior to the start of treatment. In some embodiments, the individual does not have active or untreated central nervous system (CNS) metastases. In some embodiments, the individual has treated asymptomatic supratentorial or cerebellar CNS metastases. In some embodiments, the individual does not have metastases to the midbrain, pons, medulla, or spinal cord. In some embodiments, the individual has CNS disease and does not require corticosteroid treatment for CNS disease. In some embodiments, the individual has new asymptomatic metastases and has received radiation therapy and/or surgery for CNS metastases. In some embodiments, the individual with CNS metastases has not received stereotactic radiation within 7 days of starting treatment. In some embodiments, the individual with CNS metastases has not received whole-brain radiation within 14 days of starting treatment. In some embodiments, the individual does not have leptomeningeal disease. In some embodiments, the individual does not have uncontrolled tumor pain. In some embodiments, the individual does not have uncontrolled pleural effusion. In some embodiments, the individual does not have uncontrolled pericardial effusion. In some embodiments, the individual does not have malignancies other than NSCLC within 5 years prior to the start of treatment.

[0256] In some embodiments, the individual has measurable NSCLC (*e.g.*, Stage IV non-squamous NSCLC), according to/as defined by RECIST v1.1 criteria (*see, e.g.*, Eisenhauer *et al.* (2009) "New response evaluation criteria in solid tumors: Revised RECIST guideline (version 1.1)." *Eur. J. Cancer.* 45: 228-247). In some embodiments, the individual has not received prior treatment with a CD137 agonist or an immune checkpoint blockade therapy, *e.g.*, including, without limitation, an anti-PD-1 antibody or an anti-PD-L1 antibody. In some embodiments, the patient has received prior treatment with an anti-cytotoxic T-lymphocyte associated antigen 4 (CTLA-4), wherein the treatment occurred at least 6 week prior to the start of a treatment described herein.

[0257] Any of the PD-1 axis binding antagonists, antimetabolites, and platinum agents known in the art or described herein may be used in the methods. In some embodiments, the PD-1 axis binding antagonist is atezolizumab, the antimetabolite is pemetrexed and/or the platinum agent is carboplatin or cisplatin.

[0258] In some embodiments, the atezolizumab is administered at a dose of 1200 mg, the carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min, and the pemetrexed is administered at a dose of 500 mg/m².

[0259] In some embodiments, the atezolizumab is administered at a dose of 1200 mg, the cisplatin and is administered at a dose of 75 mg/m², and the pemetrexed and is administered at a dose of 500 mg/m².

[0260] In some embodiments, treatment comprises an induction phase and a maintenance phase (or “maintenance therapy”). In some embodiments, the induction phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, the antimetabolite (*e.g.*, pemetrexed) at a dose of 500mg/m² on Day 1, and the platinum agent (*e.g.*, carboplatin) at a dose sufficient to achieve an initial target Area Under the Curve (AUC) of 6 mg/mL/min on Day 1 of each 21-day cycle for Cycles 1-4. In some embodiments, the maintenance phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, and the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 4.

[0261] In some embodiments, the induction phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1, and the platinum agent (*e.g.*, cisplatin) at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4. In some embodiments, the maintenance phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, and the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 4.

[0262] In some embodiments, the induction phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1, and the platinum agent (*e.g.*, carboplatin) at a dose sufficient to achieve an initial target Area Under the Curve (AUC) of 6 mg/mL/min on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the maintenance phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, and the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 6.

[0263] In some embodiments, the induction phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1, and the platinum agent (*e.g.*, cisplatin) at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the maintenance phase comprises administering the PD-1 axis binding antagonist (*e.g.*, an anti-PD-L1 antibody such as atezolizumab) at a dose of 1200 mg on Day 1, and the antimetabolite (*e.g.*, pemetrexed) at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 6.

[0264] Exemplary dosing and administration schedules that comprises an induction cycle and a maintenance cycle are provided in *Tables 4A* and *4B* below:

Table 4A: Exemplary Dosing and Administration Schedule

	Induction Phase	Maintenance Phase
Drugs (listed in order of administration)	Cycles 1-4	> Cycle 4*
	Day 1	Day 1
1) anti-PD-L1 Ab (atezolizumab)	1200 mg	1200 mg
2) antimetabolite (pemetrexed)	500 mg/m ²	500 mg/m ²
3) platinum agent (carboplatin or cisplatin)	Carboplatin: AUC = 6 [†] OR Cisplatin: 75 mg/m ²	

* 21-day cycles

† mg/ml/min

Table 4B: Exemplary Dosing and Administration Schedule

	Induction Phase	Maintenance Phase
Drugs (listed in order of administration)	Cycles 1-6	> Cycle 6*
	Day 1	Day 1
1) anti-PD-L1 Ab (atezolizumab)	1200 mg	1200 mg
2) antimetabolite (pemetrexed)	500 mg/m ²	500 mg/m ²
3) platinum agent (carboplatin or cisplatin)	Carboplatin: AUC = 6 [†] OR Cisplatin: 75 mg/m ²	

* 21-day cycles

† mg/ml/min

[0265] In some embodiments, the 1200 mg dose of atezolizumab is equivalent to an average body weight-based dose of 15 mg/kg. In some embodiments the dose of carboplatin needed to

achieve an AUC of 6 mg/mL/min is calculated according to the Calvert formula (*see, e.g., Calvert et al. (1989) "Carboplatin dosage: prospective evaluation of a simple formula based on renal function." J. Clin. Oncol. 7: 1748-56; van Warmerdam et al. (1995) J. Cancer Res. Clin. Oncol. 121(8): 478-486*). For further details, *see* Example 1 below.

[0266] In some embodiments, the progression free survival (PFS) of the individual is measured according to RECIST v1.1 criteria, as described in Eisenhauer *et al.* (2009) "New response evaluation criteria in solid tumors: Revised RECIST guideline (Version 1.1)." *Eur J Cancer. 45:228-47*). In some embodiments, PFS is measured as the period of time from the start of treatment to the first occurrence of disease progression as determined by RECIST v1.1 criteria. In some embodiments, PFS is measured as the time from the start of treatment to the time of death. In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months (including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months. In some embodiments, the treatment increases the PFS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.,* Stage IV non-squamous non-small cell lung cancer) who received treatment with an antimetabolite (*e.g.,* pemetrexed) and a platinum agent (*e.g.,* carboplatin or cisplatin).

[0267] In some embodiments, overall survival (OS) is measured as the period of time from the start of treatment to death. In some embodiments, the treatment increases the OS of the individual by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5, 17, 17.5, 18, 18.5, 19, 19.5, or 20 months (including any range in between these values). In some embodiments, the treatment increases the OS of the individual by at least about 18.1 months. In some embodiments, the treatment increases the OS of the individual by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 months (including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.,* Stage IV non-squamous non-small cell lung cancer) who received treatment with an antimetabolite (*e.g.,* pemetrexed) and a platinum agent (*e.g.,* carboplatin or cisplatin).

[0268] In some embodiments, the individual is human.

[0269] In some embodiments, the individual has cancer that is resistant (has been demonstrated to be resistant) to one or more PD-1 axis antagonists. In some embodiments, resistance to PD-1 axis antagonist includes recurrence of cancer or refractory cancer. Recurrence may refer to the reappearance of cancer, in the original site or a new site, after treatment. In some embodiments, resistance to PD-1 axis antagonist includes progression of the cancer during treatment with the PD-1 axis antagonist. In some embodiments, resistance to PD-1 axis antagonist includes cancer that does

not response to treatment. The cancer may be resistant at the beginning of treatment or it may become resistant during treatment. In some embodiments, the cancer is at early stage or at late stage.

[0270] In another aspect, the individual has cancer that expresses (has been shown to express *e.g.*, in a diagnostic test) PD-L1 biomarker. In some embodiments, the patient's cancer expresses low PD-L1 biomarker. In some embodiments, the patient's cancer expresses high PD-L1 biomarker. In some embodiments of any of the methods, assays and/or kits, the PD-L1 biomarker is absent from the sample when it comprises 0% of the sample.

[0271] In some embodiments of any of the methods, assays and/or kits, the PD-L1 biomarker is present in the sample when it comprises more than 0% of the sample. In some embodiments, the PD-L1 biomarker is present in at least 1% of the sample. In some embodiments, the PD-L1 biomarker is present in at least 5% of the sample. In some embodiments, the PD-L1 biomarker is present in at least 10% of the sample.

[0272] In some embodiments of any of the methods, assays and/or kits, the PD-L1 biomarker is detected in the sample using a method selected from the group consisting of FACS, Western blot, ELISA, immunoprecipitation, immunohistochemistry, immunofluorescence, radioimmunoassay, dot blotting, immunodetection methods, HPLC, surface plasmon resonance, optical spectroscopy, mass spectrometry, HPLC, qPCR, RT-qPCR, multiplex qPCR or RT-qPCR, RNA-seq, microarray analysis, SAGE, MassARRAY technique, and FISH, and combinations thereof.

[0273] In some embodiments of any of the methods, assays and/or kits, the PD-L1 biomarker is detected in the sample by protein expression. In some embodiments, protein expression is determined by immunohistochemistry (IHC). In some embodiments, the PD-L1 biomarker is detected using an anti-PD-L1 antibody. In some embodiments, the PD-L1 biomarker is detected as a weak staining intensity by IHC. In some embodiments, the PD-L1 biomarker is detected as a moderate staining intensity by IHC. In some embodiments, the PD-L1 biomarker is detected as a strong staining intensity by IHC. In some embodiments, the PD-L1 biomarker is detected on tumor cells, tumor infiltrating immune cells, stromal cells and any combinations thereof. In some embodiments, the staining is membrane staining, cytoplasmic staining or combinations thereof.

[0274] In some embodiments, the PD-L1 biomarker is detected using an anti-PD-L1 rabbit monoclonal primary antibody. In some embodiments, the PD-L1 is detected in a formalin-fixed paraffin-embedded sample. In some embodiments, the anti-PD-L1 rabbit monoclonal primary antibody is detected with a secondary antibody comprising a detectable label. In some embodiments, the assay used to detect the PD-L1 is the VENTANA PD-L1 (SP142) assay (commercially available from VENTANA®), which is described in greater detail in the Examples.

[0275] In some embodiments of any of the methods, assays and/or kits, the absence of the PD-L1 biomarker is detected as absent or no staining in the sample. In some embodiments of any of the

methods, assays and/or kits, the presence of the PD-L1 biomarker is detected as any staining in the sample.

[0276] The PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) may be administered in any order. For example, PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) may be administered sequentially (at different times) or concurrently (at the same time). In some embodiments, PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) are in separate compositions. In some embodiments, one or more (or all three) of the PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) are in the same composition.

[0277] The PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) may be administered by the same route of administration or by different routes of administration. In some embodiments, the PD-1 axis binding antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the antimetabolite (such as pemetrexed) is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, the platinum agent (such as carboplatin or cisplatin) is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some embodiments, PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) are administered via intravenous infusion. An effective amount of the PD-1 axis binding antagonist (such as atezolizumab), the antimetabolite (such as pemetrexed), and the platinum agent (such as carboplatin or cisplatin) may be administered for prevention or treatment of disease.

[0278] In some embodiments, provided is a method of treating lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer (NSCLC) in an individual (*e.g.*, an individual who is treatment-naïve for Stage IV non-squamous non-small cell lung cancer (NSCLC)) that comprises administering to the individual an effective amount of atezolizumab, pemetrexed, and carboplatin, wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the carboplatin at a dose sufficient to achieve an initial target Area Under the Curve (AUC) of 6 mg/mL/min of each 21-day cycle for Cycles 1-4. In some embodiments, the

maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1 and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 4.

[0279] In some embodiments, provided is a method of treating lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer (NSCLC) in an individual (*e.g.*, an individual who is treatment-naïve for Stage IV non-squamous non-small cell lung cancer (NSCLC)) that comprises administering to the individual an effective amount of atezolizumab, pemetrexed, and cisplatin wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, pemetrexed at a dose of 500 mg/m² on Day 1, and the cisplatin at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4. In some embodiments, the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1 and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 4.

[0280] In some embodiments, provided is a method of treating lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer (NSCLC) in an individual (*e.g.*, an individual who is treatment-naïve for Stage IV non-squamous non-small cell lung cancer (NSCLC)) that comprises administering to the individual an effective amount of atezolizumab, pemetrexed, and carboplatin wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the carboplatin at a dose sufficient to achieve an initial target Area Under the Curve (AUC) of 6 mg/mL/min on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1 and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 6.

[0281] In some embodiments, provided is a method of treating lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer (NSCLC) in an individual (*e.g.*, an individual who is treatment-naïve for Stage IV non-squamous non-small cell lung cancer (NSCLC)) that comprises administering to the individual an effective amount of atezolizumab, pemetrexed, and cisplatin wherein the administering comprises an induction phase and a maintenance phase, wherein the induction phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1, the pemetrexed at a dose of 500 mg/m² on Day 1, and the cisplatin at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6. In some embodiments, the maintenance phase comprises administering the atezolizumab at a dose of 1200 mg on Day 1 and the pemetrexed at a dose of 500 mg/m² on Day 1 of each 21-day cycle following Cycle 6.

[0282] In some embodiments, the method extends the PFS of the individual (*e.g.*, by at least about any one of 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, or 10 months, including any range in between these values) and/or the OS of the individual (*e.g.*, by at least about any one of 14, 14.5, 15, 15.5, 16, 16.5,

17, 17.5, 18, 18.5, 19, 19.5, or 20 months, including any range in between these values). In some embodiments, the treatment increases the progression free survival (PFS) of the individual by at least about 7.6 months and/or OS of the individual by at least about 18.1 months. In some embodiments, the method extends the PFS of the individual (*e.g.*, by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4 months, including any range in between these values) and/or the OS of the individual (*e.g.*, by at least about any one of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 months, including any range in between these values), as compared to an individual having lung cancer (such as non-small cell lung cancer, *e.g.*, Stage IV non-squamous non-small cell lung cancer) who received treatment with a platinum agent (*e.g.*, carboplatin or cisplatin) and an antimetabolite (*e.g.*, pemetrexed).

[0283] In some embodiments, the administration of atezolizumab is followed by the administration of pemetrexed, and the administration of pemetrexed is followed by the administration of carboplatin or cisplatin on Day 1 of each 21-day cycle for Cycles 1-4, *e.g.*, as shown in *Table 4A* above. In some embodiments, the administration of atezolizumab is followed by the administration of pemetrexed, and the administration of pemetrexed is followed by the administration of carboplatin or cisplatin on Day 1 of each 21-day cycle for Cycles 1-6, *e.g.*, as shown in *Table 4B* above.

[0284] In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes), the pemetrexed is administered intravenously over a period of about 10 minutes, and the carboplatin is administered intravenously over a period of about 30-60 minutes on Day 1 for each 21-Day cycle in Cycles 1-4. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes), the pemetrexed is administered intravenously over a period of about 10 minutes, and the carboplatin is administered intravenously over a period of about 30-60 minutes on Day 1 for Cycle 1, and the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes), the pemetrexed is administered intravenously over a period of about 10 minutes, and the carboplatin is administered intravenously over a period of about 30-60 minutes, on Day 1 of Cycles 2-4. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes), and the pemetrexed is administered intravenously over approximately 10 minutes, on Day 1 of each 21-day cycle following Cycle 4. In some embodiments, the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 4.

[0285] In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 1-2 hours on Day 1 for each 21-Day cycle in Cycles 1-4. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 1-2 hours on Day 1 for Cycle 1, and the atezolizumab is administered intravenously over 30 ($\pm$

10 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 30-60 minutes on Day 1 of Cycles 2-4. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1 and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 4. In some embodiments, the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) on Day 1 and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 4.

[0286] In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the carboplatin is administered intravenously over a period of about 30-60 minutes on Day 1 for each 21-Day cycle in Cycles 1-6. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the carboplatin is administered intravenously over a period of about 30-60 minutes on Day 1 for Cycle 1, and the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the carboplatin is administered intravenously over a period of about 30-60 minutes on Day 1 of Cycles 2-6. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1 and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 6. In some embodiments, the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) on Day 1 and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 6.

[0287] In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 1-2 hours on Day 1 for each 21-Day cycle in Cycles 1-6. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 1-2 hours on Day 1 for Cycle 1, and the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) on Day 1, the pemetrexed is administered intravenously over a period of about 10 minutes on Day 1, and the cisplatin is administered intravenously over a period of about 30-60 minutes on Day 1 of Cycles 2-6. In some embodiments, the atezolizumab is administered intravenously over 60 ($\pm$ 15 minutes) on Day 1 and the pemetrexed is administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 6. In some embodiments, the atezolizumab is administered intravenously over 30 ($\pm$ 10 minutes) on Day 1 and the pemetrexed is

administered intravenously over approximately 10 minutes on Day 1 of each 21-day cycle following Cycle 6.

[0288] As a general proposition, the therapeutically effective amount of the antibody administered to human will be in the range of about 0.01 to about 50 mg/kg of patient body weight whether by one or more administrations. In some embodiments, the antibody used is about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In some embodiments, the antibody is administered at 15 mg/kg. However, other dosage regimens may be useful. In one embodiment, an anti-PDL1 antibody described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (*e.g.*, 2 or 3 doses), such as infusions. The dose of the antibody administered in a combination treatment may be reduced as compared to a single treatment. The progress of this therapy is easily monitored by conventional techniques.

[0289] In some embodiments, the methods may further comprise an additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy. In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, etc.). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation.

[0290] In some embodiments, the additional therapy comprises CT-011 (also known as Pidilizumab or MDV9300; CAS Registry No. 1036730-42-3; CureTech/Medivation). CT-011, also known as hBAT or hBAT-1, is an antibody described in WO2009/101611. In some embodiments, the additional therapeutic comprises an antibody that comprises a heavy chain and a light chain sequence, wherein:

(a) the heavy chain comprises the amino acid sequence:

QVQLVQSGSELKKPGASVKISCKASGYTFTNYGMNWVRQAPGQGLQWMGWINTDSGESTY
AEEFKGRFVFSLDTSVNTAYLQITSLTAEDTGMVFCVRVGYDALDYWGQGTLVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS
VVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
HNHYTQKSLSLSPGK (SEQ ID NO:19), and

(b) the light chain comprises the amino acid sequence:

EIVLTQSPSSLSASVGDRVTITCSARSSVSYMHWFQKPGKAPKLWIYRTSNLASGVPSRFSG
SGSGTSYCLTINSLPEDFATYYCQQRSSFPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:20).

[0291] In some embodiments, the additional therapeutic antibody comprises the six HVR sequences from SEQ ID NO: 19 and SEQ ID NO: 20 (*e.g.*, the three heavy chain HVRs from SEQ ID NO: 19 and the three light chain HVRs from SEQ ID NO: 20). In some embodiments, the additional therapeutic antibody comprises the heavy chain variable domain from SEQ ID NO: 19 and the light chain variable domain from SEQ ID NO: 20.

[0292] Other additional therapeutic antibodies contemplated for use herein include, without limitation, alemtuzumab (Campath), bevacizumab (AVASTIN®, Genentech); cetuximab (ERBITUX®, Imclone); panitumumab (VECTIBIX®, Amgen), rituximab (RITUXAN®, Genentech/Biogen Idec), pertuzumab (OMNITARG®, 2C4, Genentech), trastuzumab (HERCEPTIN®, Genentech), tositumomab (Bexxar, Corixa), the antibody drug conjugate gemtuzumab ozogamicin (MYLOTARG®, Wyeth), apolizumab, aselizumab, atlizumab, bapineuzumab, bivatuzumab mertansine, cantuzumab mertansine, cedelizumab, certolizumab pegol, cidfusituzumab, cidtuzumab, daclizumab, eculizumab, efalizumab, epratuzumab, erlizumab, felvizumab, fontolizumab, gemtuzumab ozogamicin, inotuzumab ozogamicin, ipilimumab, labetuzumab, lintuzumab, matuzumab, mepolizumab, motavizumab, motovizumab, natalizumab, nimotuzumab, nolovizumab, numavizumab, ocrelizumab, omalizumab, palivizumab, pascolizumab, pecfusituzumab, pectuzumab, pexelizumab, ralivizumab, ranibizumab, reslivizumab, reslizumab, resyvizumab, rovelizumab, ruplizumab, sibrotuzumab, sipilizumab, sontuzumab, tacatuzumab tetraxetan, tadocizumab, talizumab, tefibazumab, tocilizumab, toralizumab, tucotuzumab celmoleukin, tucusituzumab, umavizumab, urtoxazumab, ustekinumab, visilizumab, and the anti-interleukin-12 (ABT-874/J695, Wyeth Research and Abbott Laboratories).

[0293] In some embodiments, the additional therapy is therapy targeting PI3K/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. In some embodiments, the additional therapy is CTLA-4 (also known as CD152), *e.g.*, a blocking antibody, ipilimumab (also known as MDX-010, MDX-101, or Yervoy®), tremelimumab (also

known as ticilimumab or CP-675,206), an antagonist directed against B7-H3 (also known as CD276), *e.g.*, a blocking antibody, MGA271, an antagonist directed against a TGF beta, *e.g.*, metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), or LY2157299, a treatment comprising adoptive transfer of a T cell (*e.g.*, a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR), a treatment comprising adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, *e.g.*, a dominant-negative TGF beta type II receptor, a treatment comprising a HERCREEM protocol (*see*, *e.g.*, ClinicalTrials.gov Identifier NCT00889954), an agonist directed against CD137 (also known as TNFRSF9, 4-1BB, or ILA), *e.g.*, an activating antibody, urelumab (also known as BMS-663513), an agonist directed against CD40, *e.g.*, an activating antibody, CP-870893, an agonist directed against OX40 (also known as CD134), *e.g.*, an activating antibody, administered in conjunction with a different anti-OX40 antibody (*e.g.*, AgonOX), an agonist directed against CD27, *e.g.*, an activating antibody, CDX-1127, indoleamine-2,3-dioxygenase (IDO), 1-methyl-D-tryptophan (also known as 1-D-MT), an antibody-drug conjugate (in some embodiments, comprising mertansine or monomethyl auristatin E (MMAE)), an anti-NaPi2b antibody-MMAE conjugate (also known as DNIB0600A or RG7599), trastuzumab emtansine (also known as T-DM1, ado-trastuzumab emtansine, or KADCYLA®, Genentech), DMUC5754A, an antibody-drug conjugate targeting the endothelin B receptor (EDNBR), *e.g.*, an antibody directed against EDNBR conjugated with MMAE, an angiogenesis inhibitor, an antibody directed against a VEGF, *e.g.*, VEGF-A, bevacizumab (also known as AVASTIN®, Genentech), an antibody directed against angiopoietin 2 (also known as Ang2), MEDI3617, an antineoplastic agent, an agent targeting CSF-1R (also known as M-CSFR or CD115), anti-CSF-1R (also known as IMC-CS4), an interferon, for example interferon alpha or interferon gamma, Roferon-A, GM-CSF (also known as recombinant human granulocyte macrophage colony stimulating factor, rhu GM-CSF, sargramostim, or Leukine®), IL-2 (also known as aldesleukin or Proleukin®), IL-12, an antibody targeting CD20 (in some embodiments, the antibody targeting CD20 is obinutuzumab (also known as GA101 or Gazyva®) or rituximab), an antibody targeting GITR (in some embodiments, the antibody targeting GITR is TRX518), in conjunction with a cancer vaccine (in some embodiments, the cancer vaccine is a peptide cancer vaccine, which in some embodiments is a personalized peptide vaccine; in some embodiments the peptide cancer vaccine is a multivalent long peptide, a multi-peptide, a peptide cocktail, a hybrid peptide, or a peptide-pulsed dendritic cell vaccine (*see, e.g.*, Yamada *et al.*, Cancer Sci, 104:14-21, 2013)), in conjunction with an adjuvant, a TLR agonist, *e.g.*, Poly-ICLC (also known as Hiltonol®), LPS, MPL, or CpG ODN, tumor necrosis factor (TNF) alpha, IL-1, HMGB1, an IL-10 antagonist, an IL-4 antagonist, an IL-13 antagonist, an HVEM antagonist, an ICOS agonist, *e.g.*, by administration of ICOS-L, or an agonistic antibody directed against ICOS, a treatment targeting CX3CL1, a treatment targeting CXCL10, a treatment targeting CCL5, an LFA-1 or ICAM1 agonist, a Selectin agonist, a targeted therapy, an inhibitor of B-Raf, vemurafenib (also known as Zelboraf®,

dabrafenib (also known as Tafinlar®), erlotinib (also known as Tarceva®), an inhibitor of a MEK, such as MEK1 (also known as MAP2K1) or MEK2 (also known as MAP2K2), cobimetinib (also known as GDC-0973 or XL-518), trametinib (also known as Mekinist®), an inhibitor of K-Ras, an inhibitor of c-Met, onartuzumab (also known as MetMab), an inhibitor of Alk, AF802 (also known as CH5424802 or alectinib), an inhibitor of a phosphatidylinositol 3-kinase (PI3K), BKM120, idelalisib (also known as GS-1101 or CAL-101), perifosine (also known as KRX-0401), an Akt, MK2206, GSK690693, GDC-0941, an inhibitor of mTOR, sirolimus (also known as rapamycin), temsirolimus (also known as CCI-779 or Torisel®), everolimus (also known as RAD001), ridaforolimus (also known as AP-23573, MK-8669, or deforolimus), OSI-027, AZD8055, INK128, a dual PI3K/mTOR inhibitor, XL765, GDC-0980, BEZ235 (also known as NVP-BEZ235), BGT226, GSK2126458, PF-04691502, PF-05212384 (also known as PKI-587). The additional therapy may be one or more of the chemotherapeutic agents described herein.

IX. Methods of Detection and Diagnosis

[0294] In some embodiments, the sample is obtained prior to treatment with a PD-1 axis binding antagonist (*e.g.*, atezolizumab), a platinum agent (*e.g.*, carboplatin or cisplatin), and an antimetabolite (*e.g.*, pemetrexed). In some embodiments, the tissue sample is formalin fixed and paraffin embedded, archival, fresh or frozen.

[0295] In some embodiments, the sample is whole blood. In some embodiments, the whole blood comprises immune cells, circulating tumor cells and any combinations thereof.

[0296] Presence and/or expression levels/amount of a biomarker (*e.g.*, PD-L1) can be determined qualitatively and/or quantitatively based on any suitable criterion known in the art, including but not limited to DNA, mRNA, cDNA, proteins, protein fragments and/or gene copy number. In certain embodiments, presence and/or expression levels/amount of a biomarker in a first sample is increased or elevated as compared to presence/absence and/or expression levels/amount in a second sample. In certain embodiments, presence/absence and/or expression levels/amount of a biomarker in a first sample is decreased or reduced as compared to presence and/or expression levels/amount in a second sample. In certain embodiments, the second sample is a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. Additional disclosures for determining presence/absence and/or expression levels/amount of a gene are described herein.

[0297] In some embodiments of any of the methods, elevated expression refers to an overall increase of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, the elevated expression refers to the increase in expression level/amount of a biomarker in the sample wherein the increase is at least about any of 1.5X, 1.75X, 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X,

25X, 50X, 75X, or 100X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In some embodiments, elevated expression refers to an overall increase of greater than about 1.5 fold, about 1.75 fold, about 2 fold, about 2.25 fold, about 2.5 fold, about 2.75 fold, about 3.0 fold, or about 3.25 fold as compared to a reference sample, reference cell, reference tissue, control sample, control cell, control tissue, or internal control (*e.g.*, housekeeping gene).

[0298] In some embodiments of any of the methods, reduced expression refers to an overall reduction of about any of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or greater, in the level of biomarker (*e.g.*, protein or nucleic acid (*e.g.*, gene or mRNA)), detected by standard art known methods such as those described herein, as compared to a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue. In certain embodiments, reduced expression refers to the decrease in expression level/amount of a biomarker in the sample wherein the decrease is at least about any of 0.9X, 0.8X, 0.7X, 0.6X, 0.5X, 0.4X, 0.3X, 0.2X, 0.1X, 0.05X, or 0.01X the expression level/amount of the respective biomarker in a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue.

[0299] Presence and/or expression level/amount of various biomarkers in a sample can be analyzed by a number of methodologies, many of which are known in the art and understood by the skilled artisan, including, but not limited to, immunohistochemistry (“IHC”), Western blot analysis, immunoprecipitation, molecular binding assays, ELISA, ELIFA, fluorescence activated cell sorting (“FACS”), MassARRAY, proteomics, quantitative blood based assays (as for example Serum ELISA), biochemical enzymatic activity assays, in situ hybridization, Southern analysis, Northern analysis, whole genome sequencing, polymerase chain reaction (“PCR”) including quantitative real time PCR (“qRT-PCR”) and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like), RNA-Seq, FISH, microarray analysis, gene expression profiling, and/or serial analysis of gene expression (“SAGE”), as well as any one of the wide variety of assays that can be performed by protein, gene, and/or tissue array analysis. Typical protocols for evaluating the status of genes and gene products are found, for example in Ausubel *et al.*, eds., 1995, Current Protocols In Molecular Biology, Units 2 (Northern Blotting), 4 (Southern Blotting), 15 (Immunoblotting) and 18 (PCR Analysis). Multiplexed immunoassays such as those available from Rules Based Medicine or Meso Scale Discovery (“MSD”) may also be used.

[0300] In some embodiments, presence and/or expression level/amount of a biomarker is determined using a method comprising: (a) performing gene expression profiling, PCR (such as rtPCR or qRT-PCR), RNA-seq, microarray analysis, SAGE, MassARRAY technique, or FISH on a sample (such as a subject cancer sample); and b) determining presence and/or expression level/amount of a biomarker in the sample. In some embodiments, the microarray method comprises the use of a microarray chip having one or more nucleic acid molecules that can hybridize under

stringent conditions to a nucleic acid molecule encoding a gene mentioned above or having one or more polypeptides (such as peptides or antibodies) that can bind to one or more of the proteins encoded by the genes mentioned above. In one embodiment, the PCR method is qRT-PCR. In one embodiment, the PCR method is multiplex-PCR. In some embodiments, gene expression is measured by microarray. In some embodiments, gene expression is measured by qRT-PCR. In some embodiments, expression is measured by multiplex-PCR.

[0301] Methods for the evaluation of mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as in situ hybridization using labeled riboprobes specific for the one or more genes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for one or more of the genes, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like).

[0302] Samples from mammals can be conveniently assayed for mRNAs using Northern, dot blot or PCR analysis. In addition, such methods can include one or more steps that allow one to determine the levels of target mRNA in a biological sample (*e.g.*, by simultaneously examining the levels a comparative control mRNA sequence of a “housekeeping” gene such as an actin family member). Optionally, the sequence of the amplified target cDNA can be determined.

[0303] Optional methods include protocols which examine or detect mRNAs, such as target mRNAs, in a tissue or cell sample by microarray technologies. Using nucleic acid microarrays, test and control mRNA samples from test and control tissue samples are reverse transcribed and labeled to generate cDNA probes. The probes are then hybridized to an array of nucleic acids immobilized on a solid support. The array is configured such that the sequence and position of each member of the array is known. For example, a selection of genes whose expression correlates with increased or reduced clinical benefit of anti-angiogenic therapy may be arrayed on a solid support. Hybridization of a labeled probe with a particular array member indicates that the sample from which the probe was derived expresses that gene.

[0304] According to some embodiments, presence and/or expression level/amount is measured by observing protein expression levels of an aforementioned gene. In certain embodiments, the method comprises contacting the biological sample with antibodies to a biomarker (*e.g.*, anti-PD-L1 antibodies) described herein under conditions permissive for binding of the biomarker, and detecting whether a complex is formed between the antibodies and biomarker. Such method may be an *in vitro* or *in vivo* method. In one embodiment, an antibody is used to select subjects eligible for therapy with PD-L1 axis binding antagonist *e.g.*, a biomarker for selection of individuals.

[0305] In certain embodiments, the presence and/or expression level/amount of biomarker proteins in a sample is examined using IHC and staining protocols. IHC staining of tissue sections has been shown to be a reliable method of determining or detecting presence of proteins in a sample.

In some embodiments of any of the methods, assays and/or kits, the PD-L1 biomarker is PD-L1. In some embodiments, PD-L1 is detected by immunohistochemistry. In some embodiments, elevated expression of a PD-L1 biomarker in a sample from an individual is elevated protein expression and, in further embodiments, is determined using IHC. In one embodiment, expression level of biomarker is determined using a method comprising: (a) performing IHC analysis of a sample (such as a subject cancer sample) with an antibody; and b) determining expression level of a biomarker in the sample. In some embodiments, IHC staining intensity is determined relative to a reference. In some embodiments, the reference is a reference value. In some embodiments, the reference is a reference sample (*e.g.*, control cell line staining sample or tissue sample from non-cancerous patient).

[0306] IHC may be performed in combination with additional techniques such as morphological staining and/or fluorescence in-situ hybridization. Two general methods of IHC are available; direct and indirect assays. According to the first assay, binding of antibody to the target antigen is determined directly. This direct assay uses a labeled reagent, such as a fluorescent tag or an enzyme-labeled primary antibody, which can be visualized without further antibody interaction. In a typical indirect assay, unconjugated primary antibody binds to the antigen and then a labeled secondary antibody binds to the primary antibody. Where the secondary antibody is conjugated to an enzymatic label, a chromogenic or fluorogenic substrate is added to provide visualization of the antigen. Signal amplification occurs because several secondary antibodies may react with different epitopes on the primary antibody.

[0307] The primary and/or secondary antibody used for IHC typically will be labeled with a detectable moiety. Numerous labels are available which can be generally grouped into the following categories: (a) Radioisotopes, such as ^{35}S , ^{14}C , ^{125}I , ^3H , and ^{131}I ; (b) colloidal gold particles; (c) fluorescent labels including, but are not limited to, rare earth chelates (europium chelates), Texas Red, rhodamine, fluorescein, dansyl, Lissamine, umbelliferone, phycocrytherin, phycocyanin, or commercially available fluorophores such SPECTRUM ORANGE7 and SPECTRUM GREEN7 and/or derivatives of any one or more of the above; (d) various enzyme-substrate labels are available and U.S. Patent No. 4,275,149 provides a review of some of these. Examples of enzymatic labels include luciferases (*e.g.*, firefly luciferase and bacterial luciferase; U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases (*e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like.

[0308] Examples of enzyme-substrate combinations include, for example, horseradish peroxidase (HRPO) with hydrogen peroxide as a substrate; alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and β -D-galactosidase (β -D-Gal) with a chromogenic substrate

(*e.g.*, p-nitrophenyl- β -D-galactosidase) or fluorogenic substrate (*e.g.*, 4-methylumbelliferyl- β -D-galactosidase). For a general review of these, *see* U.S. Patent Nos. 4,275,149 and 4,318,980.

[0309] In some embodiments of any of the methods, PD-L1 is detected by immunohistochemistry using an anti- PD-L1 diagnostic antibody (*i.e.*, primary antibody). In some embodiments, the PD-L1 diagnostic antibody specifically binds human PD-L1. In some embodiments, the PD-L1 diagnostic antibody is a nonhuman antibody. In some embodiments, the PD-L1 diagnostic antibody is a rat, mouse, or rabbit antibody. In some embodiments, the PD-L1 diagnostic antibody is a monoclonal antibody. In some embodiments, the PD-L1 diagnostic antibody is directly labeled.

[0310] Specimens thus prepared may be mounted and coverslipped. Slide evaluation is then determined, *e.g.*, using a microscope, and staining intensity criteria, routinely used in the art, may be employed. In one embodiment, it is understood that when cells and/or tissue from a tumor is examined using IHC, staining is generally determined or assessed in tumor cell and/or tissue (as opposed to stromal or surrounding tissue that may be present in the sample). In some embodiments, it is understood that when cells and/or tissue from a tumor is examined using IHC, staining includes determining or assessing in tumor infiltrating immune cells, including intratumoral or peritumoral immune cells.

[0311] In some embodiments, PDL1 expression is evaluated on a tumor or tumor sample. As used herein, a tumor or tumor sample may encompass part or all of the tumor area occupied by tumor cells. In some embodiments, a tumor or tumor sample may further encompass tumor area occupied by tumor associated intratumoral cells and/or tumor associated stroma (*e.g.*, contiguous peri-tumoral desmoplastic stroma). Tumor associated intratumoral cells and/or tumor associated stroma may include areas of immune infiltrates (*e.g.*, tumor infiltrating immune cells as described herein) immediately adjacent to and/or contiguous with the main tumor mass. In some embodiments, PDL1 expression is evaluated on tumor cells. In some embodiments, PDL1 expression is evaluated on immune cells within the tumor area as described above, such as tumor infiltrating immune cells.

[0312] In alternative methods, the sample may be contacted with an antibody specific for said biomarker under conditions sufficient for an antibody-biomarker complex to form, and then detecting said complex. The presence of the biomarker may be detected in a number of ways, such as by Western blotting and ELISA procedures for assaying a wide variety of tissues and samples, including plasma or serum. A wide range of immunoassay techniques using such an assay format are available, *see, e.g.*, U.S. Pat. Nos. 4,016,043, 4,424,279 and 4,018,653. These include both single-site and two-site or “sandwich” assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labeled antibody to a target biomarker.

[0313] Presence and/or expression level/amount of a selected biomarker in a tissue or cell sample may also be examined by way of functional or activity-based assays. For instance, if the biomarker is

an enzyme, one may conduct assays known in the art to determine or detect the presence of the given enzymatic activity in the tissue or cell sample.

[0314] In certain embodiments, the samples are normalized for both differences in the amount of the biomarker assayed and variability in the quality of the samples used, and variability between assay runs. Such normalization may be accomplished by detecting and incorporating the expression of certain normalizing biomarkers, including well known housekeeping genes. Alternatively, normalization can be based on the mean or median signal of all of the assayed genes or a large subset thereof (global normalization approach). On a gene-by-gene basis, measured normalized amount of a subject tumor mRNA or protein is compared to the amount found in a reference set. Normalized expression levels for each mRNA or protein per tested tumor per subject can be expressed as a percentage of the expression level measured in the reference set. The presence and/or expression level/amount measured in a particular subject sample to be analyzed will fall at some percentile within this range, which can be determined by methods well known in the art.

[0315] In one embodiment, the sample is a clinical sample. In another embodiment, the sample is used in a diagnostic assay. In some embodiments, the sample is obtained from a primary or metastatic tumor. Tissue biopsy is often used to obtain a representative piece of tumor tissue. Alternatively, tumor cells can be obtained indirectly in the form of tissues or fluids that are known or thought to contain the tumor cells of interest. For instance, samples of lung cancer lesions may be obtained by resection, bronchoscopy, fine needle aspiration, bronchial brushings, or from sputum, pleural fluid or blood. Genes or gene products can be detected from cancer or tumor tissue or from other body samples such as urine, sputum, serum or plasma. The same techniques discussed above for detection of target genes or gene products in cancerous samples can be applied to other body samples. Cancer cells may be sloughed off from cancer lesions and appear in such body samples. By screening such body samples, a simple early diagnosis can be achieved for these cancers. In addition, the progress of therapy can be monitored more easily by testing such body samples for target genes or gene products.

[0316] In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a single sample or combined multiple samples from the same subject or individual that are obtained at one or more different time points than when the test sample is obtained. For example, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained at an earlier time point from the same subject or individual than when the test sample is obtained. Such reference sample, reference cell, reference tissue, control sample, control cell, or control tissue may be useful if the reference sample is obtained during initial diagnosis of cancer and the test sample is later obtained when the cancer becomes metastatic.

[0317] In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combined multiple samples from one or more healthy

individuals who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is a combined multiple samples from one or more individuals with a disease or disorder (*e.g.*, cancer) who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from normal tissues or pooled plasma or serum samples from one or more individuals who are not the subject or individual. In certain embodiments, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is pooled RNA samples from tumor tissues or pooled plasma or serum samples from one or more individuals with a disease or disorder (*e.g.*, cancer) who are not the subject or individual.

[0318] In some embodiments, the sample is a tissue sample from the individual. In some embodiments, the tissue sample is a tumor tissue sample (*e.g.*, biopsy tissue). In some embodiments, the tissue sample is lung tissue. In some embodiments, the tissue sample is renal tissue. In some embodiments, the tissue sample is skin tissue. In some embodiments, the tissue sample is pancreatic tissue. In some embodiments, the tissue sample is gastric tissue. In some embodiments, the tissue sample is bladder tissue. In some embodiments, the tissue sample is esophageal tissue. In some embodiments, the tissue sample is mesothelial tissue. In some embodiments, the tissue sample is breast tissue. In some embodiments, the tissue sample is thyroid tissue. In some embodiments, the tissue sample is colorectal tissue. In some embodiments, the tissue sample is head and neck tissue. In some embodiments, the tissue sample is osteosarcoma tissue. In some embodiments, the tissue sample is prostate tissue. In some embodiments, the tissue sample is ovarian tissue, HCC (liver), blood cells, lymph nodes, and/or bone/bone marrow tissue. In some embodiments, the tissue sample is colon tissue. In some embodiments, the tissue sample is endometrial tissue. In some embodiments, the tissue sample is brain tissue (*e.g.*, glioblastoma, neuroblastoma, and so forth).

[0319] In some embodiments, a tumor tissue sample (the term “tumor sample” is used interchangeably herein) may encompass part or all of the tumor area occupied by tumor cells. In some embodiments, a tumor or tumor sample may further encompass tumor area occupied by tumor associated intratumoral cells and/or tumor associated stroma (*e.g.*, contiguous peri-tumoral desmoplastic stroma). Tumor associated intratumoral cells and/or tumor associated stroma may include areas of immune infiltrates (*e.g.*, tumor infiltrating immune cells as described herein) immediately adjacent to and/or contiguous with the main tumor mass.

[0320] In some embodiments, the PD-L1 biomarker is detected in the sample using a method selected from the group consisting of FACS, Western blot, ELISA, immunoprecipitation, immunohistochemistry, immunofluorescence, radioimmunoassay, dot blotting, immunodetection methods, HPLC, surface plasmon resonance, optical spectroscopy, mass spectrometry, HPLC, qPCR, RT-qPCR, multiplex qPCR or RT-qPCR, RNA-seq, microarray analysis, SAGE, MassARRAY technique, and FISH, and combinations thereof. In some embodiments, the PD-L1 biomarker is

detected using FACS analysis. In some embodiments, the PD-L1 biomarker is PD-L1. In some embodiments, the PD-L1 expression is detected in blood samples. In some embodiments, the PD-L1 expression is detected on circulating immune cells in blood samples. In some embodiments, the circulating immune cell is a CD3+/CD8+ T cell. In some embodiments, prior to analysis, the immune cells are isolated from the blood samples. Any suitable method to isolate/enrich such population of cells may be used including, but not limited to, cell sorting. In some embodiments, the PD-L1 expression is elevated in samples from individuals that respond to treatment with an inhibitor of the PD-L1/PD-1 axis pathway, such as an anti-PD-L1 antibody. In some embodiments, the PD-L1 expression is elevated on the circulating immune cells, such as the CD3+/CD8+ T cells, in blood samples.

[0321] In some embodiments, the anti-PD-L1 rabbit monoclonal primary antibody is detected with a secondary antibody comprising a detectable label. In some embodiments, the assay used to detect the PD-L1 is the VENTANA PD-L1 (SP142) assay (commercially available from VENTANA®), which is described in greater detail in the Examples.

[0322] Certain aspects of the present disclosure relate to measurement of the expression level of one or more genes or one or more proteins in a sample. In some embodiments, a sample may include leukocytes. In some embodiments, the sample may be a peripheral blood sample (*e.g.*, from a patient having a tumor). In some embodiments, the sample is a tumor sample. A tumor sample may include cancer cells, lymphocytes, leukocytes, stroma, blood vessels, connective tissue, basal lamina, and any other cell type in association with the tumor. In some embodiments, the sample is a tumor tissue sample containing tumor-infiltrating leukocytes. In some embodiments, the sample may be processed to separate or isolate one or more cell types (*e.g.*, leukocytes). In some embodiments, the sample may be used without separating or isolating cell types.

[0323] A tumor sample may be obtained from a subject by any method known in the art, including without limitation a biopsy, endoscopy, or surgical procedure. In some embodiments, a tumor sample may be prepared by methods such as freezing, fixation (*e.g.*, by using formalin or a similar fixative), and/or embedding in paraffin wax. In some embodiments, a tumor sample may be sectioned. In some embodiments, a fresh tumor sample (*i.e.*, one that has not been prepared by the methods described above) may be used. In some embodiments, a tumor sample may be prepared by incubation in a solution to preserve mRNA and/or protein integrity.

[0324] In some embodiments, the sample may be a peripheral blood sample. A peripheral blood sample may include white blood cells, PBMCs, and the like. Any technique known in the art for isolating leukocytes from a peripheral blood sample may be used. For example, a blood sample may be drawn, red blood cells may be lysed, and a white blood cell pellet may be isolated and used for the sample. In another example, density gradient separation may be used to separate leukocytes (*e.g.*, PBMCs) from red blood cells. In some embodiments, a fresh peripheral blood sample (*i.e.*, one that

has not been prepared by the methods described above) may be used. In some embodiments, a peripheral blood sample may be prepared by incubation in a solution to preserve mRNA and/or protein integrity.

[0325] In some embodiments, responsiveness to treatment may refer to any one or more of: extending survival (including overall survival and progression free survival); resulting in an objective response (including a complete response or a partial response); or improving signs or symptoms of cancer. In some embodiments, responsiveness may refer to improvement of one or more factors according to the published set of RECIST guidelines for determining the status of a tumor in a cancer patient, *i.e.*, responding, stabilizing, or progressing. For a more detailed discussion of these guidelines, *see* Eisenhauer *et al.*, *Eur J Cancer* 2009;45: 228–47; Topalian *et al.*, *N Engl J Med* 2012;366:2443–54; Wolchok *et al.*, *Clin Can Res* 2009;15:7412–20; and Therasse, P., *et al. J. Natl. Cancer Inst.* 92:205-16 (2000). A responsive subject may refer to a subject whose cancer(s) show improvement, *e.g.*, according to one or more factors based on RECIST criteria. A non-responsive subject may refer to a subject whose cancer(s) do not show improvement, *e.g.*, according to one or more factors based on RECIST criteria.

[0326] Conventional response criteria may not be adequate to characterize the anti-tumor activity of immunotherapeutic agents, which can produce delayed responses that may be preceded by initial apparent radiological progression, including the appearance of new lesions. Therefore, modified response criteria have been developed that account for the possible appearance of new lesions and allow radiological progression to be confirmed at a subsequent assessment. Accordingly, in some embodiments, responsiveness may refer to improvement of one or more factors according to immune-related response criteria² (irRC). *See, e.g.*, Wolchok *et al.*, *Clin Can Res* 2009; 15:7412 – 20. In some embodiments, new lesions are added into the defined tumor burden and followed, *e.g.*, for radiological progression at a subsequent assessment. In some embodiments, presence of non-target lesions are included in assessment of complete response and not included in assessment of radiological progression. In some embodiments, radiological progression may be determined only on the basis of measurable disease and/or may be confirmed by a consecutive assessment ≥ 4 weeks from the date first documented.

[0327] In some embodiments, responsiveness may include immune activation. In some embodiments, responsiveness may include treatment efficacy. In some embodiments, responsiveness may include immune activation and treatment efficacy.

X. Articles of Manufacture or Kits

[0328] In another embodiment of the invention, an article of manufacture or a kit is provided comprising a PD-1 axis binding antagonist (such as atezolizumab) and/or a platinum agent (such as carboplatin or cisplatin) and/or an antimetabolite (such as pemetrexed). In some embodiments, the article of manufacture or kit further comprises package insert comprising instructions for using the

PD-1 axis binding antagonist in conjunction with the platinum agent (such as carboplatin or cisplatin) and the antimetabolite (such as pemetrexed) to treat or delay progression of cancer (*e.g.*, lung cancer, such as non-small cell lung cancer (NSCLC), including Stage IV non-squamous NSCLC) in an individual or to enhance immune function of an individual having cancer (*e.g.*, lung cancer, such as NSCLC, including Stage IV non-squamous NSCLC). Any of the PD-1 axis binding antagonists, platinum agents known in the art may be included in the article of manufacture or kits. In some embodiments, the kit comprises atezolizumab, carboplatin or cisplatin, and pemetrexed.

[0329] In some embodiments, the PD-1 axis binding antagonist (such as atezolizumab), the platinum agent (such as carboplatin or cisplatin) and the antimetabolite (such as pemetrexed) are in the same container or separate containers. Suitable containers include, for example, bottles, vials, bags and syringes. The container may be formed from a variety of materials such as glass, plastic (such as polyvinyl chloride or polyolefin), or metal alloy (such as stainless steel or hastelloy). In some embodiments, the container holds the formulation and the label on, or associated with, the container may indicate directions for use. The article of manufacture or 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 use. In some embodiments, the article of manufacture further includes one or more of another agent (*e.g.*, a chemotherapeutic agent, and anti-neoplastic agent). Suitable containers for the one or more agent include, for example, bottles, vials, bags and syringes.

[0330] The specification is considered to be sufficient to enable one skilled in the art to practice the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0331] The present disclosure will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the 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: A Phase III, open-label, randomized study of atezolizumab in combination with carboplatin + pemetrexed or cisplatin + pemetrexed compared with carboplatin + pemetrexed or cisplatin + pemetrexed in patients who are chemotherapy-naïve and have Stage IV non-squamous non-small cell lung cancer (NSCLC)

[0332] This study was designed to evaluate the efficacy, safety, and pharmacokinetics of atezolizumab in combination with carboplatin + pemetrexed or cisplatin + pemetrexed compared with carboplatin + pemetrexed or cisplatin + pemetrexed in patients who are chemotherapy-naïve and have Stage IV non-squamous non-small cell lung cancer (NSCLC). Specific objectives and corresponding endpoints for the study are outlined below.

Study Objectives

[0333] The co-primary efficacy objectives of this study were the following:

- To evaluate the efficacy of atezolizumab + carboplatin + pemetrexed compared with carboplatin + pemetrexed and the efficacy of atezolizumab + cisplatin + pemetrexed compared with cisplatin + pemetrexed in the intent-to-treat (ITT) population as measured by investigator-assessed progression-free survival (PFS) according to RECIST v1.1 (*see, e.g., Eisenhauer et al. (2009) "New response evaluation criteria in solid tumors: Revised RECIST guideline (Version 1.1)." Eur J Cancer. 45:228-47*) or death from any cause, whichever occurred first.
- To evaluate the efficacy of atezolizumab + carboplatin + pemetrexed compared with carboplatin + pemetrexed and the efficacy of atezolizumab + cisplatin + pemetrexed compared with cisplatin + pemetrexed as measured by overall survival (OS), defined as the time from randomization to death from any cause.

[0334] The secondary efficacy objectives of this study were the following:

- To evaluate the efficacy of atezolizumab + carboplatin + pemetrexed compared with carboplatin + pemetrexed and the efficacy of atezolizumab + cisplatin + pemetrexed compared with cisplatin + pemetrexed as measured by objective response rate (ORR), defined as partial response (PR) or complete response (CR) according to RECIST v1.1.
- To evaluate the efficacy of atezolizumab + carboplatin + pemetrexed compared with carboplatin + pemetrexed and the efficacy of atezolizumab + cisplatin + pemetrexed compared with cisplatin + pemetrexed as measured by investigator-assessed duration of response (DOR) according to RECIST v1.1.
- To evaluate the OS rate at 1 and 2 years.
- To determine the impact of atezolizumab as measured by the change from baseline in patient-reported lung cancer symptoms of cough, dyspnea, chest pain, or arm/shoulder pain, using the European Organization for the Research and Treatment of Cancer (EORTC) Quality-of-Life Questionnaire–Core 30 (QLQ-C30) and the supplemental lung cancer module (QLQ-LC13).
- TTD (time to deterioration) in patient lung cancer symptoms, defined as time from randomization to deterioration (10 point change) on each of the EORTC QLQ-30 and EORTC QLQ-LC13.
- To determine the impact of atezolizumab as measured by the change from baseline in patient-reported lung cancer symptoms (chest pain, dyspnea, and cough) scores using the Symptoms in Lung Cancer (SILC) scale symptom severity scores.

[0335] The safety objectives for this study were the following:

- To evaluate the safety and tolerability of atezolizumab + carboplatin + pemetrexed or atezolizumab + cisplatin + pemetrexed, as measured by the incidence, nature, and severity

of adverse events graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v 4.0.

- To evaluate changes in vital signs, physical findings, and clinical laboratory results during and following study treatment administration. To evaluate the safety and tolerability of atezolizumab when given in combination with carboplatin + pemetrexed or in combination with cisplatin + pemetrexed or as maintenance therapy with pemetrexed alone
- To evaluate the incidence and titers of anti-therapeutic antibodies (ATAs) against atezolizumab and to explore the potential relationship of the immunogenicity response with pharmacokinetics, safety, and efficacy.

[0336] The pharmacokinetic objectives for this study are:

- To characterize the pharmacokinetics of atezolizumab when given in combination with carboplatin + pemetrexed, cisplatin + pemetrexed, or pemetrexed alone.
- To characterize the pharmacokinetics of carboplatin when given in combination with atezolizumab + pemetrexed.
- To characterize the pharmacokinetics of cisplatin when given in combination with atezolizumab + pemetrexed.
- To characterize the pharmacokinetics of pemetrexed when given in combination with atezolizumab + carboplatin or in combination with atezolizumab + cisplatin
- To determine the maximum observed serum atezolizumab concentration (C_{max}) after infusion (Arm A).
- To determine the minimum observed serum atezolizumab concentration (C_{min}) prior to infusion at selected cycles, at treatment discontinuation, and at 120 days (± 30 days) after the last dose of atezolizumab (Arm A).
- To determine plasma concentrations for carboplatin or cisplatin (Arm A).
- To determine plasma concentrations for pemetrexed (Arm A)

[0337] The exploratory objectives for this study are:

- To evaluate the progression-free survival (PFS) rate at 6-month and 1-year landmark time points.
- To evaluate the overall survival (OS) rate at 3 years in each treatment arm.
- To evaluate the efficacy of atezolizumab as measured by OS and investigator-assessed PFS according to RECIST v1.1 in subgroups based on demographic and baseline characteristics.
- To evaluate the efficacy of atezolizumab as measured by milestone survival.
- To evaluate the relationship between biomarkers in tumors and blood (including but not limited to programmed death–ligand 1 (PD-L1), programmed death-1 (PD-1), somatic mutations and others), as defined by immunohistochemistry (IHC), quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR), next-generation sequencing, and/or other methods and measures of efficacy.
- To assess predictive, prognostic, and pharmacodynamic exploratory biomarkers in archival and/or fresh tumor tissue and blood and their association with disease status, mechanisms of resistance, and/or response to study treatment.
- To evaluate status of PD-L1–, immune- and NSCLC-related and other exploratory biomarkers in archival and/or fresh tumor tissues, and blood (or blood derivatives) collected before, during, or after treatment with atezolizumab or at progression and

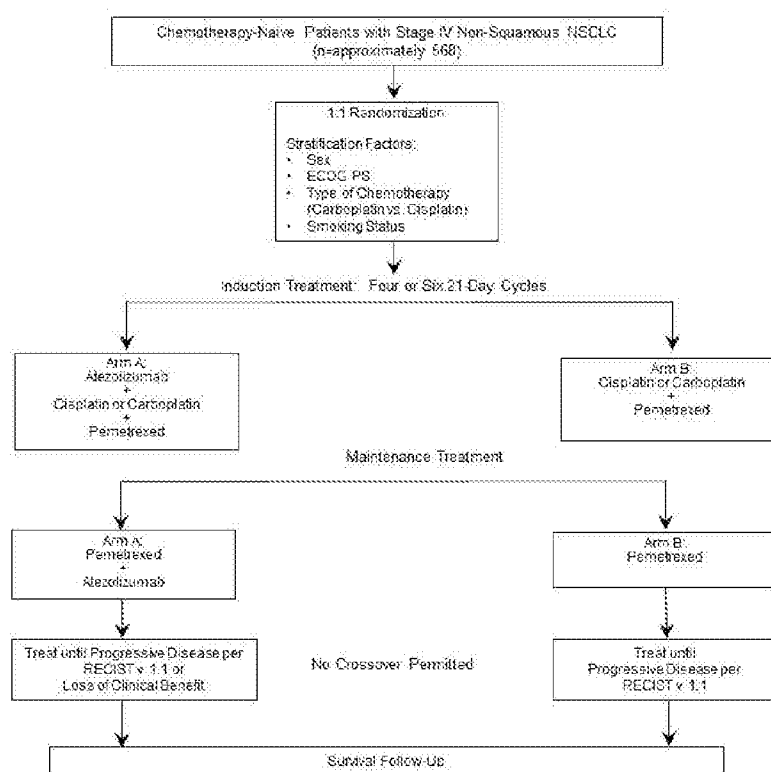
association with disease status and/or response to atezolizumab in combination with chemotherapy.

- To evaluate and compare patient's health status as assessed by the EuroQoL 5 Dimensions 5-Level (EQ-5D-5L) questionnaire to generate utility scores for use in economic models for reimbursement.
- To determine the impact of atezolizumab in each of the treatment comparisons as measured by change from baseline in patient-reported outcomes (PROs) of health-related quality of life, lung cancer-related symptoms, and health status as assessed by the European Organization for Research and Treatment of Cancer Quality of Life Questionnaires EORTC QLQ-C30 and QLQ-LC13

Study Design

[0338] Described below are the details of a randomized, Phase III, multicenter, open-label study designed to evaluate the safety and efficacy of (a) atezolizumab + carboplatin + pemetrexed compared with treatment with carboplatin + pemetrexed and (b) atezolizumab + cisplatin + pemetrexed compared with treatment with cisplatin + pemetrexed in patients who are chemotherapy-naïve and have Stage IV non-squamous NSCLC. *Scheme 1* below illustrates the study design:

Scheme 1



[0339] In *Scheme 1* above, ECOG PS refers to “Eastern Cooperative Oncology Group performance status,” NSCLC refers to “non-small cell lung cancer,” and RECIST v1.1 refers to “Response Evaluation Criteria in Solid Tumors, Version 1.1.”

[0340] This study enrolled approximately 568 patients across all sites in a global enrollment phase. Patients were stratified by sex (male vs. female), smoking status (never vs. current and/or former), ECOG (*i.e.*, Eastern Cooperative Oncology Group) performance status (0 vs. 1), and chemotherapy regimen (carboplatin vs. cisplatin), and were then randomized 1:1 to receive one of the following treatment regimens as shown in *Table 5* below. Further details regarding ECOG performance status are provided in Oken *et al.* (1982) *Am J Clin Oncol.* 5: 649-655).

Table 5: Treatment Arms

Treatment Arm	Induction Phase (Four or Six 21-Day Cycles)	Maintenance Phase (21-Day Cycles)
A	atezolizumab + carboplatin + pemetrexed or atezolizumab + cisplatin + pemetrexed	atezolizumab + pemetrexed
B	carboplatin + pemetrexed or cisplatin + pemetrexed	pemetrexed

[0341] The induction phase was administered on a 21-day cycle for four or six cycles. The number of cycles of induction treatment (*i.e.*, four or six) was at the discretion of the investigator and was determined and documented prior to randomization. Induction treatment was administered on a 21-day cycle until the following occurred (whichever occurred first): 1) administration of four or six cycles, 2) unacceptable toxicity, or 3) documented disease progression.

[0342] Following the induction phase, patients who had not experienced progression or unacceptable toxicity continued maintenance therapy with either atezolizumab + pemetrexed (Arm A), or pemetrexed alone (Arm B). Patients randomized to either Arm A or B continued treatment with atezolizumab + pemetrexed maintenance or pemetrexed maintenance until progressive disease, unacceptable toxicity, or death. During induction or maintenance phase, patients randomized to Arm A continued treatment with atezolizumab beyond progressive disease by RECIST v1.1, provided they experienced clinical benefit as assessed by the investigator as described below:

[0343] **For Treatment Arm A:** During treatment (induction or maintenance), patients who showed evidence of clinical benefit were permitted to continue atezolizumab after RECIST v1.1 for progressive disease were met if they met all of the following criteria:

- Evidence of clinical benefit as assessed by the investigator.

- Absence of symptoms and signs (including worsening of laboratory values [*e.g.*, new or worsening hypercalcemia]) indicating unequivocal progression of disease.
- No decline in ECOG performance status that could be attributed to disease progression.
- Absence of tumor progression at critical anatomical sites (*e.g.*, leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions.
- Patients must have provided written consent to acknowledge deferring other treatment options in favor of continuing study treatment at the time of initial progression.

[0344] Treatment with chemotherapy (both in Arms A and B) was discontinued in all patients who exhibit evidence of progressive disease by RECIST v1.1

[0345] The dosing and administration schedule for the treatment regimens in Arm A of *Table 5* are provided in *Tables 6A* and *6B* below:

Table 6A: Dosing and Administration Schedule for Treatment with 4-Cycle Induction Phase

	Induction Phase	Maintenance Phase
Drugs (listed in order of administration)	Cycles 1-4	> Cycle 4*
	Day 1	Day 1
1) anti-PD-L1 Ab (atezolizumab)	1200 mg	1200 mg
2) antimetabolite (pemetrexed)	500 mg/m ²	500 mg/m ²
3) platinum agent (carboplatin or cisplatin)	Carboplatin: AUC = 6 [†] OR Cisplatin: 75 mg/m ²	

* 21-day cycles

† mg/ml/min

Table 6B: Dosing and Administration Schedule for Treatment with 6-Cycle Induction Phase

	Induction Phase	Maintenance Phase
Drugs (listed in order of administration)	Cycles 1-6	> Cycle 6*
	Day 1	Day 1
1) anti-PD-L1 Ab (atezolizumab)	1200 mg	1200 mg

2) antimetabolite (pemetrexed)	500 mg/m ²	500 mg/m ²
3) platinum agent (carboplatin or cisplatin)	Carboplatin: AUC = 6 [†] OR Cisplatin: 75 mg/m ²	

* 21-day cycles

† mg/ml/min

[0346] Patients underwent tumor assessments at baseline and every 6 weeks (± 7 days) for the first 48 weeks following Cycle 1, Day 1, regardless of dose delays. After the completion of the Week 48 tumor assessment, tumor assessment was required every 9 weeks (± 7 days) thereafter, regardless of treatment dose delays. Patients underwent tumor assessments until radiographic disease progression per RECIST v1.1 or loss of clinical benefit (for atezolizumab-treated only patients who continued treatment after radiographic disease progression according to RECIST v1.1), withdrawal of consent, study termination by Sponsor, or death, whichever occurred first. Patients who discontinued treatment for reasons other than radiographic disease progression (*e.g.*, toxicity) continued scheduled tumor assessments until radiographic disease progression per RECIST v1.1 or loss of clinical benefit (for atezolizumab-treated patients who continued treatment after radiographic disease progression according to RECIST v1.1), withdrawal of consent, study termination by Sponsor, or death, whichever occurred first, regardless of whether patients started a new anti-cancer therapy.

[0347] If clinically feasible, it was recommended that patients undergo a tumor biopsy sample collection at the time of radiographic disease progression. These data were used to explore whether radiographic findings were consistent with the presence of a tumor. Additionally, these data were analyzed to evaluate the association between changes in tumor tissue and clinical outcome and to further understand the potential mechanisms of progression and resistance to atezolizumab as compared with such mechanisms after treatment with chemotherapy alone. This exploratory biomarker evaluation was not used for any treatment-related decisions.

[0348] Patients who continued treatment after radiographic disease progression per RECIST v1.1 continued to undergo tumor assessments every 6 weeks (± 7 days), or sooner if symptomatic deterioration occurred. For these patients, tumor assessments continued every 6 weeks (± 7 days), regardless of time in the study, until study treatment was discontinued.

[0349] Patients who discontinued treatment for reasons other than radiographic disease progression per RECIST v1.1 (*e.g.*, toxicity, symptomatic deterioration) continued scheduled tumor assessments at the same frequency as would have been followed if the patient had remained on study treatment (*i.e.*, every 6 weeks (± 7 days) for 48 weeks following Cycle 1, Day 1 and then every 9 weeks (± 7 days) thereafter, regardless of treatment dose delays) until radiographic disease

progression per RECIST v1.1, withdrawal of consent, study termination by Sponsor, or death, whichever occurred first.

[0350] Patients who started a new anti-cancer therapy in the absence of radiographic disease progression per RECIST v1.1 continued scheduled tumor assessments until radiographic disease progression per RECIST v1.1 (or loss of clinical benefit for atezolizumab-treated patients who had continued treatment with atezolizumab after radiographic disease progression according to RECIST v1.1), withdrawal of consent, death, or study termination by the Sponsor, whichever occurred first.

[0351] Tumor assessments for patients who were treated with atezolizumab who continued to experience clinical benefit, despite evidence of radiographic progression, was continued as per the schedule listed above.

Patients

[0352] Patients were eligible for participation in this study if they were chemotherapy-naive and had Stage IV non-squamous NSCLC.

Inclusion Criteria

[0353] The key inclusion criteria included: an age 18 years or older; ECOG performance status of 0 or 1; histologically or cytologically confirmed Stage IV non-squamous NSCLC (per the Union Internationale contre le Cancer/American Joint Committee on Cancer staging system, 7th edition; Detterbeck *et al.* (2009) "The new lung cancer staging system" *Chest* 136 :260-71); patients with tumors of mixed non-small cell histology (*i.e.*, squamous and non-squamous) were eligible if the major histological component appeared to be non-squamous; no prior treatment for Stage IV non-squamous NSCLC; patients who had received prior neo-adjuvant, adjuvant chemotherapy, radiotherapy, or chemoradiotherapy with curative intent for non-metastatic disease must have experienced a treatment-free interval of at least 6 months from randomization since the last dose of chemotherapy and/or radiotherapy; patients with a history of treated asymptomatic CNS metastases were eligible only if (a) the metastases were supratentorial and/or cerebellar (*i.e.*, no metastases to midbrain, pons, medulla or spinal cord); (b) the patients had no ongoing requirement for corticosteroids as therapy for CNS disease, (c) patients had no stereotactic radiation within 7 days or whole-brain radiation within 14 days prior to randomization, (d) patients had no evidence of interim progression between the completion of CNS-directed therapy and the screening radiograph study; patients with new asymptomatic CNS metastases detected at the screening scan must have received radiation therapy and/or surgery for CNS metastases. Patients with new asymptomatic CNS metastases detected at the screening scan must have received radiation therapy and/or surgery for CNS metastases. Following treatment, these patients may have been eligible without the need for an additional brain scan prior to randomization, if all other criteria were met. Eligible patients were to

have demonstrated measurable disease, as defined by RECIST v1.1 (previously irradiated lesions were only considered as measurable disease if disease progression had been unequivocally documented at that site since radiation and the previously irradiated lesion was not the only site of disease); adequate hematologic and end organ function, defined by the following laboratory test results obtained within 14 days prior to randomization:

- ANC ≥ 1500 cells/ μ L without granulocyte colony-stimulating factor support.
- Lymphocyte count $\geq 500/\mu$ L.
- Platelet count $\geq 100,000/\mu$ L without transfusion.
- Hemoglobin ≥ 9.0 g/dL. (Patients were permitted to be transfused to meet this criterion.)
- INR or aPTT $\leq 1.5 \times$ upper limit of normal (ULN). (This applied only to patients who were not receiving therapeutic anticoagulation; patients receiving therapeutic anticoagulation were required to be on a stable dose.)
- AST, ALT, and alkaline phosphatase $\leq 2.5 \times$ ULN, with the following exceptions:
 - Patients with documented liver metastases: AST and/or ALT $\leq 5 \times$ ULN.
 - Patients with documented liver or bone metastases: alkaline phosphatase $\leq 5 \times$ ULN.
- Serum bilirubin $\leq 1.25 \times$ ULN. (Patients with known Gilbert disease who had serum bilirubin level $\leq 3 \times$ ULN were enrolled.)
- Calculated creatinine clearance (CRCL) ≥ 45 mL/min or, if using cisplatin, calculated CRCL must be ≥ 60 mL/min.

[0354] Patients were encouraged to submit a pre-treatment tumor tissue sample (if available). If tumor tissue was not available (*e.g.*, depleted for prior diagnostic testing), patients were still eligible. If tumor tissue was available, a representative formalin-fixed paraffin-embedded (FFPE) tumor specimen in paraffin block or unstained, freshly cut, serial sections (preferably at least 10) from an FFPE tumor specimen was preferred. If 10 sections were not available, fewer could be submitted. If FFPE specimens described above were not available, any type of specimens (including fine-needle aspiration, cell pellet specimens [*e.g.*, from pleural effusion], and lavage samples) were also acceptable. Specimens were accompanied by an associated pathology report. Any available tumor tissue sample were required to be submitted before or within 4 weeks after enrollment.

Exclusion Criteria

[0355] Key exclusion criteria included: patients with a sensitizing mutation in the EGFR gene or an ALK fusion oncogene; treatment with any other investigational agent with therapeutic intent within 28 days prior to randomization; active or untreated CNS metastases as determined by computed

tomography (CT) or magnetic resonance imaging (MRI) evaluation during screening and prior radiographic assessments; spinal cord compression not definitively treated with surgery and/or radiation or previously diagnosed and treated spinal cord compression without evidence that disease has been clinically stable for ≥ 2 weeks prior to randomization; leptomeningeal disease; uncontrolled tumor-related pain (patients requiring pain medication must have been receiving a stable regimen at study entry; symptomatic lesions amenable to palliative radiotherapy (*e.g.*, bone metastases or metastases causing nerve impingement) were required to be treated prior to randomization (patients should be recovered from the effects of radiation and there is no required minimum recovery period). Patients with asymptomatic metastatic lesions whose further growth would likely cause functional deficits or intractable pain (*e.g.*, epidural metastasis that is not currently associated with spinal compression) were considered for loco-regional therapy, if appropriate, prior to randomization). Exclusion criteria also included: uncontrolled pleural effusion, pericardial effusion, or ascites requiring recurrent drainage procedures (once monthly or more frequently, but patients with indwelling catheters (*e.g.*, PleurX[®]) were allowed regardless of drainage frequency); uncontrolled or symptomatic hypercalcemia (> 1.5 mmol/L ionized calcium or calcium > 12 mg/dL or corrected serum calcium $> \text{ULN}$; patients who were receiving denosumab prior to randomization were, if eligible, required to discontinue its use and replace it with a bisphosphonate while in the study); malignancies other than SCLC within 5 years prior to randomization, with the exception of those with a negligible risk of metastasis or death (*e.g.*, expected 5-year OS $> 90\%$) treated with expected curative outcome (such as adequately treated carcinoma in situ of the cervix, basal or squamous-cell skin cancer, localized prostate cancer treated surgically with curative intent, ductal carcinoma in situ treated surgically with curative intent); known tumor PD-L1 expression status as determined by an IHC assay from other clinical studies (*e.g.*, patients whose PD-L1 expression status was determined during screening for entry into a study with anti-PD-1 or anti-PD-L1 antibodies but were not eligible were excluded); women who were pregnant, lactating, or intending to become pregnant during the study; history of autoimmune disease, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, vasculitis, or glomerulonephritis (patients with a history of autoimmune-related hypothyroidism on thyroid replacement hormone therapy were eligible; patients with controlled Type I diabetes mellitus on an insulin regimen were eligible); history of idiopathic pulmonary fibrosis, organizing pneumonia (*e.g.*, bronchiolitis obliterans), drug-induced pneumonitis, idiopathic pneumonitis, or evidence of active pneumonitis on screening chest CT scan. (History of radiation pneumonitis in the radiation field (fibrosis) was permitted); positive test result for HIV; patients with active hepatitis B (chronic or acute; defined as having a positive hepatitis B surface antigen [HBsAg] test result at screening) or hepatitis C virus

(HCV); active tuberculosis; severe infections within 4 weeks prior to randomization, including but not limited to hospitalization for complications of infection, bacteremia, or severe pneumonia; therapeutic oral or IV antibiotics within 2 weeks prior to randomization (patients receiving prophylactic antibiotics (*e.g.*, for prevention of a urinary tract infection or to prevent chronic obstructive pulmonary disease exacerbation) were eligible); significant cardiovascular disease, such as New York Heart Association cardiac disease (Class II or greater), myocardial infarction, or cerebrovascular accident within 3 months prior to randomization, unstable arrhythmias, or unstable angina. (Patients with known coronary artery disease, congestive heart failure not meeting the above criteria, or left ventricular ejection fraction < 50% were required to be on a stable medical regimen that is optimized in the opinion of the treating physician, in consultation with a cardiologist if appropriate); major surgical procedure other than for diagnosis within 28 days prior to randomization or anticipation of need for a major surgical procedure during the course of the study; prior allogeneic bone marrow transplantation or solid organ transplant; any other diseases, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicated the use of an investigational drug or that may have affected the interpretation of the results or rendered the patient at high risk for treatment complications; patients with illnesses or conditions that interfere with their capacity to understand, follow, and/or comply with study procedures; treatment with any other investigational agent with therapeutic intent within 28 days prior to randomization; administration of a live, attenuated vaccine within 4 weeks before randomization or anticipation that such a live attenuated vaccine would be required during the study; prior treatment with *EGFR* inhibitors or *ALK* inhibitors; any approved anti-cancer therapy, including hormonal therapy within 21 days prior to initiation of study treatment; prior treatment with CD137 agonists or immune checkpoint blockade therapies, anti-PD-1, and anti-PD-L1 therapeutic antibodies. (Patients who have had prior anti-cytotoxic T lymphocyte associated antigen 4 (CTLA-4) treatment were eligible for enrollment, provided that the last dose of anti CTLA-4 at least 6 weeks prior to randomization, and that the patients had no history of severe immune-related adverse effects from anti CTLA-4 (NCI CTCAE Grade 3 and 4).) Key exclusion criteria also included: treatment with any other investigational agent with therapeutic intent within 28 days prior to randomization, treatment with systemic immunostimulatory agents (including, but not limited to interferons and interleukin 2) within 4 weeks or 5 half-lives of the drug, whichever is longer, prior to randomization (prior treatment with cancer vaccines was allowed); treatment with systemic immunosuppressive medications (including, but not limited to, corticosteroids, cyclophosphamide, azathioprine, methotrexate, thalidomide, and anti-tumor necrosis factor [anti-TNF] agents) within 2 weeks prior to randomization. Patients who had received acute, low-dose (≤ 10 mg oral prednisone or equivalent), systemic immunosuppressant medications were eligible to be enrolled in the study. Additionally, the use of corticosteroids (≤ 10 mg oral prednisone or equivalent) for chronic obstructive pulmonary disease,

mineralocorticoids (*e.g.*, fludrocortisone) for patients with orthostatic hypotension, and low-dose supplemental corticosteroids for adrenocortical insufficiency was allowed). Patients were excluded if they had a history of severe allergic, anaphylactic, or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins; known hypersensitivity or allergy to biopharmaceuticals produced in Chinese hamster ovary cells or any component of the atezolizumab formulation; and history of allergic reactions to carboplatin, cisplatin, or other platinum-containing compounds; patients with hearing impairment (cisplatin); Grade ≥ 2 peripheral neuropathy as defined by NCI CTCAE v4.0 (cisplatin); creatinine clearance level < 60 mL/min for cisplatin or < 45 mL/min for carboplatin

Method of Treatment

[0356] 568 patients were randomized (1:1) to receive treatment with atezolizumab + carboplatin + pemetrexed or atezolizumab + cisplatin + pemetrexed (Arm A) or carboplatin + pemetrexed or cisplatin + pemetrexed (Arm B). (The details of Treatment Arms A and B are shown above in **Table 5**).

[0357] During the induction phase, a chemotherapy cycle counted toward the prespecified number of induction chemotherapy cycles (4 or 6) as long as at least one chemotherapy component had been administered at least once during a 21-day cycle. Cycles in which no chemotherapy component is given did not count toward the total number of induction chemotherapy cycles.

[0358] Patients who experienced no further clinical benefit (for patients enrolled into Arm A) or disease progression (for patients enrolled into Arm B) at any time during the induction phase discontinued all study treatment. In the absence of the above criteria, after the 4 or 6-cycle induction phase, patients began maintenance therapy (atezolizumab + pemetrexed in Arm A or pemetrexed in Arm B).

[0359] During treatment (induction or maintenance), Arm A patients who showed evidence of clinical benefit were permitted to continue atezolizumab after RECIST v1.1 for progressive disease were met. However, treatment with chemotherapy was discontinued.

[0360] Patients received anti-emetics and IV hydration for platinum-pemetrexed treatments according to the local standard of care and manufacturer's instruction. However, due to their immunomodulatory effects, premedication with steroids were limited when clinically feasible. In addition, in the event of pemetrexed-related skin rash, topical steroid use was recommended as front-line treatment whenever it was clinically feasible. **Table 7** below lists the premedication for pemetrexed. **Table 8** below lists the infusion times for treatment administration for pemetrexed + platinum during the induction and maintenance phases.

Table 7: Premedication for Pemetrexed

Premedication	Dose and Route	Timing
Folic acid	350–1000 µg PO	Once daily beginning at least 5–7 days before Cycle 1, Day 1 and continuing until 3 weeks after discontinuation of pemetrexed
Vitamin B12	1000 µg IM	q9w beginning Cycle 1, Day 1 and continuing until 3 weeks after discontinuation of pemetrexed
Dexamethasone (suggested)	4 mg PO	Twice daily the day before, the day of, and the day after pemetrexed administration

IM = intramuscular

PO = oral

Q9w = every 9 weeks.

Table 8: Treatment Regimen for Pemetrexed + Platinum-Based Chemotherapy

	Study Drug	Dose and Route	Induction Period (Four or Six Cycles)	Maintenance Period (Until PD)
1	Pemetrexed	500 mg/m ² IV	Over approximately 10 minutes on Day 1 q3w	Over approximately 10 minutes on Day 1 q3w
AND				
2	Carboplatin	AUC 6 IV	Over approximately 30–60 minutes on Day 1 q3W	Not applicable
	OR			
	Cisplatin	75 mg/m ²	Over 1–2 hours on Day 1 q3w	Not applicable

AUC = area under the concentration–time curve

IV = intravenous

PD = progressive disease

Q3w = every 3 weeks.

[0361] 578 patients were randomized (1:1) to receive treatment with atezolizumab + pemetrexed + carboplatin or cisplatin (Arm A) or pemetrexed + carboplatin or cisplatin (Arm B). (The details of Treatment Arms A and B are shown above in *Tables 5, 6A, and 6B*). Patient demographics and baseline characteristics are shown in *Tables 9A and 9B* below.

Table 9A: Patient Demographics and Baseline Characteristics

Demographics/Baseline		ITT	
		ARM A	ARM B
Age (yrs)	<65	153 (52.4%)	167 (58.4%)
Sex IxRS	Male	192 (65.8%)	192 (67.1%)
Race	White	193 (66.1%)	203 (71.0%)
	Asian	71 (24.3%)	65 (22.7%)
Tobacco Use History	Never	37 (12.7%)	30 (10.5%)
Liver Metastasis	Yes	37 (12.7%)	36 (12.6%)
ECOG score	0	126 (43.2%)	114 (40.1%)
4 vs 6 cycles	4 cycles	197 (67.5%)	190 (66.4%)
IHC TC3/TC3*	Positive	25 (14.2%)	20 (12.1%)
IHC TC12/IC12*	Positive	63 (35.8%)	73 (43.5%)

*prevalence is calculated in the biomarker evaluable population

Table 9B: Baseline Characteristics

Characteristic	APP (n = 292)	PP (n = 286)	Characteristic	APP (n = 292)	PP (n = 286)
Median age (range), years	64.0 (31-85)	63.0 (33-83)	Smoking status, n (%)		
<65 years, n (%)	153 (52.4%)	167 (58.4%)	Current or former	255 (87.3%)	256 (89.5%)
Sex, male, n (%)	192 (65.8%)	192 (67.1%)	Never	37 (12.7%)	30 (10.5%)
Race, n (%) ^a			Liver metastases, n (%)	37 (12.7%)	36 (12.6%)
			PD-L1 expression, n (%) ^c	n = 176	n = 168
White	193 (66.1%)	203 (71.0%)	Negative	88 (50.0%)	75 (44.6%)
Asian	71 (24.3%)	65 (22.7%)	Positive	88 (50.0%)	93 (55.4%)
ECOG PS 0, n (%) ^b	126 (43.2%)	114 (40.1%)	PD-L1-low	63 (35.8%)	73 (43.5%)
Carboplatin, n (%)	177 (60.6%)	175 (61.1%)	PD-L1-high	25 (14.2%)	20 (11.9%)
Intended 4 cycles, n (%)	197 (67.5%)	190 (66.4%)			

ECOG, eastern cooperative oncology group; PS, performance status

^a American Indian or Alaska Native race (n = 2), Black or African American (n = 6) and Unknown race (n = 38) not included in table. ^b 2 patients has missing baseline ECOG PS. ^c PD-L1 status available in 60% of patients. PD-L1-high (TC3/IC3): patients with PD-L1 expression in ≥50% of tumor cells or ≥10% of tumor-infiltrating immune cells; PD-L1-low (TC12/IC12): patients with PD-L1 expression in ≥1% and <50% of tumor cells or ≥1% and <10% of tumor-infiltrating immune cells; and PD-L1-negative (TC0/IC0): patients with PD-L1 expression in <1% of tumor cells and <1% of tumor-infiltrating immune cells.

[0362] Patients received their first dose of study drug on the day of randomization, if possible. If this was not possible, the first dose occurred within 5 days after randomization. Atezolizumab was supplied by the Sponsor. Carboplatin, cisplatin and pemetrexed were background treatment and were considered non-investigational medicinal products (NIMPs). Carboplatin, cisplatin, and pemetrexed were used in the commercially available formulations. The atezolizumab drug product was provided as a sterile liquid in 20-mL glass vials. The vial was designed to deliver 20 mL (1200 mg) of atezolizumab solution but may have contained more than the stated volume to enable delivery of the entire 20-mL volume.

[0363] The induction phase of the study consisted of four or six cycles of atezolizumab/placebo plus chemotherapy, with each cycle being 21 days in duration. The number of cycles of induction treatment (four or six) was determined by the investigator and documented prior to randomization.

See the schema above. On Day 1 of each cycle, all eligible patients were administered study drug infusions in the following order:

Arm A: atezolizumab → pemetrexed → carboplatin or cisplatin

Arm B: pemetrexed → carboplatin or cisplatin

[0364] During the induction phase, study treatment was administered in the following manner on Day 1:

1. Atezolizumab (1200 mg, equivalent to an average body weight-based dose of 15 mg/kg), administered intravenously over 60 ($\pm$ 15) minutes (for the first infusion, with possible shortening to 30 ($\pm$ 10) minutes for subsequent infusions), followed by
2. Pemetrexed (500 mg/m²), administered intravenously over approximately 10 minutes, followed by
3. Carboplatin, administered intravenously over 30–60 minutes to achieve an initial target area under the concentration-time curve (AUC) of 6 mg/mL/min (Calvert formula dosing);

or

Cisplatin, administered intravenously over 1-2 hours at a dose of 75 mg/m².

[0365] The carboplatin dose of AUC 6 was calculated using the Calvert formula (Calvert *et al.* (1989) *J Clin Oncol* 7:1748–56):

Calvert Formula:

Total dose (mg) = (target AUC) $\times$ (glomerular filtration rate [GFR] + 25)

[0366] The GFR used in the Calvert formula to calculate AUC-based dosing was not to exceed 125 mL/min. For the purposes of this protocol, the GFR was considered to be equivalent to the creatinine clearance (CRCL). The CRCL is calculated by institutional guidelines or by the method described in Cockcroft and Gault (1976) *Nephron* 16:31–41, using the following formula:

$$\text{CRCL} = \frac{(140 - \text{age}) (\text{wt})}{72 \times \text{Scr}} (\times 0.85 \text{ if female})$$

[0367] Where: CRCL = creatinine clearance in mL/min

age = patient's age in years

wt = patient's weight in kg

Scr = serum creatinine in mg/dL

[0368] For patients with an abnormally low serum creatinine level, estimate the GFR was estimated through use of a minimum creatinine level of 0.8 mg/dL or the estimated GFR was capped at 125 mL/min. It was recommended that physicians cap the dose of carboplatin for desired exposure (AUC) to avoid potential toxicity due to overdosing. On the basis of the Calvert formula described in the carboplatin label, the maximum doses were calculated as follows:

$$\text{Maximum carboplatin dose (mg)} = \text{target AUC (mg} \times \text{min/mL)} \times (\text{GFR} + 25 \text{ mL/min})$$

[0369] The maximum dose was based on a GFR estimate that is capped at 125 mL/min for patients with normal renal function. No higher estimated GFR values were used. For a target AUC = 6, the maximum dose was $6 \times 150 = 900$ mg. For a target AUC = 5, the maximum dose was $5 \times 150 = 750$ mg. For a target AUC = 4, the maximum dose was $4 \times 150 = 600$ mg. Additional details regarding carboplatin dosing are provide in:

[www\(dot\)fda\(dot\)gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm228974.h
tm](http://www(dot)fda(dot)gov/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm228974.htm)

[0370] During the induction phase, a chemotherapy cycle counted toward the prespecified number of induction chemotherapy cycles (4 or 6) as long as at least one chemotherapy component had been administered at least once during a 21-day cycle. Cycles in which no chemotherapy was given did not count toward the total number of induction chemotherapy cycles. After the induction phase, patients began maintenance therapy with atezolizumab (*i.e.*, 1200 mg, infused IV, as described above) and pemetrexed (*i.e.*, 500 mg/m², infused IV as described above) on Day 1 of every subsequent 21-day cycle following the induction phase. *See FIG. 1* and the study schema above). No dose modifications to atezolizumab were permitted.

Permitted Therapy

[0371] Premedication with antihistamines may be administered for any atezolizumab infusions after Cycle 1. The following therapies should continue while patients are in the study:

- Oral contraceptives
- Hormone-replacement therapy
- Prophylactic or therapeutic anticoagulation therapy (such as low molecular weight heparin or warfarin at a stable dose level)
- Palliative radiotherapy (*e.g.*, treatment of known bony metastases) provided it does not interfere with the assessment of tumor target lesions (*e.g.*, the lesion being irradiated is not the only site of disease, as that would render the patient not evaluable for response by tumor assessments according to RECIST v1.1)
- It is not a requirement to withhold atezolizumab during palliative radiotherapy.

- Inactive influenza vaccinations
- Megestrol administered as an appetite stimulant
- Inhaled corticosteroids for chronic obstructive pulmonary disease
- Mineralocorticoids (*e.g.*, fludrocortisone)
- Low-dose corticosteroids for patients with orthostatic hypotension or adrenocortical insufficiency

[0372] In general, patients' care was managed with supportive therapies as clinically indicated per local standards. Patients who experienced infusion-associated symptoms may have been treated symptomatically with acetaminophen, ibuprofen, diphenhydramine, and/or famotidine or another H₂-receptor antagonist per standard practice. Serious infusion-associated events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress were managed with supportive therapies as clinically indicated (*e.g.*, supplemental oxygen and β_2 -adrenergic agonists).

Cautionary Therapy for Atezolizumab-Treated Patients

[0373] As systemic corticosteroids and TNF- α inhibitors are known to attenuate potential beneficial immunologic effects of treatment with atezolizumab. Therefore, in situations where systemic corticosteroids or TNF- α inhibitors would be routinely administered, alternatives, including antihistamines, were considered first by the treating physician. If the alternatives were not feasible, systemic corticosteroids and TNF- α inhibitors were administered at the discretion of the treating physician except in the case of patients for whom CT scans with contrast were contraindicated (*i.e.*, patients with contrast allergy or impaired renal clearance). Systemic corticosteroids are recommended, with caution at the discretion of the treating physician, for the treatment of specific adverse events when associated with atezolizumab therapy.

Prohibited Therapy

[0374] Any concomitant therapy intended for the treatment of cancer, whether health authority-approved or experimental, was prohibited for various time periods prior to starting study treatment, depending on the anti-cancer agent, and during study treatment until disease progression is documented and patient had discontinued study treatment. Prohibited concomitant therapies included, but were not limited to, chemotherapy, hormonal therapy, immunotherapy, radiotherapy, investigational agents, or herbal therapy (unless otherwise noted).

[0375] The following medications were prohibited while the patient is in the study, unless otherwise noted:

- Denosumab; patients who were receiving denosumab prior to enrollment must have been willing and eligible to receive a bisphosphonate instead.

- Any live attenuated vaccine (*e.g.*, FluMist[®]) within 4 weeks prior to randomization or during treatment or within 5 months following the last atezolizumab dose (for patients randomized to atezolizumab).
- Use of steroids to premedicate patients for whom CT scans with contrast were contraindicated (*i.e.*, patients with contrast allergy or impaired renal clearance); in such patients, non-contrast CT scans of the chest and non-contrast CT scans or MRIs of the abdomen and pelvis were performed.

[0376] The concomitant use of herbal therapies was not recommended because their pharmacokinetics, safety profiles, and potential drug-drug interactions are generally unknown. However, their use for patients in the study was allowed at the discretion of the investigator provided that there were no known interactions with any study treatment. As noted above, herbal therapies intended for the treatment of cancer were prohibited.

Tumor and Response Evaluations

[0377] Patients were closely monitored for safety and tolerability throughout the study, and were assessed for toxicity prior to each dose.

[0378] Medical histories for each patient included clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and all medications (*e.g.*, prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to the screening visit.

[0379] NSCLC cancer history included prior cancer therapies, procedures, and an assessment of tumor mutational status (*e.g.*, sensitizing *EGFR* mutation, *ALK* fusion status). For patients not previously tested for tumor mutational status, testing was required at screening. For these patients, testing was either performed locally or submitted for central evaluation during the screening period. If *EGFR* mutations or *ALK* status testing was not performed locally, additional tumor sections were required for central evaluation of the mutational status of these genes. Demographic data included age, sex, and self-reported race/ethnicity.

[0380] A complete physical examination included an evaluation of the head, eyes, ears, nose, and throat and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, genitourinary, and neurological systems. Any abnormality identified at baseline was recorded.

[0381] At subsequent visits (or as clinically indicated), limited, symptom-directed physical examinations were performed. Changes from baseline abnormalities were recorded in patient notes. New or worsened clinically significant abnormalities were recorded as adverse events.

[0382] Vital signs included measurements of temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressures while the patient is in a seated position.

Tumor and Response Evaluations

[0383] Screening assessments included computer tomography (CT) scans (with oral/IV contrast unless contraindicated) or magnetic resonance images (MRIs) of the chest and abdomen. A CT or MRI scan of the pelvis was required at screening and as clinically indicated or as per local standard-of-care at subsequent response evaluations. Spiral CT scans of the chest were obtained, if possible, but were not a requirement.

[0384] A CT (with contrast if not contraindicated) or MRI scan of the head was required at screening to evaluate CNS metastasis in all patients. An MRI scan of the brain was required to confirm or refute the diagnosis of CNS metastases at baseline in the event of an equivocal scan. Patients with active or untreated CNS metastases were not eligible for the study (*see* Exclusion Criteria).

[0385] If a CT scan for tumor assessment was performed in a positron emission tomography (PET)/CT scanner, the CT acquisition was required to be consistent with the standards for a full contrast diagnostic CT scan.

[0386] Bone scans and CT scans of the neck were also performed if clinically indicated. At the investigator's discretion, other methods of assessment of measurable disease as per RECIST v1.1 were used.

[0387] It was permissible to use tumor assessments performed as standard-of-care prior to obtaining informed consent and within 28 days of Cycle 1, Day 1 rather than repeating tests. Documentation of all known sites of disease at screening was required, and documentation reassessed at each subsequent tumor evaluation. Patients with history of irradiated brain metastases at screening were not required to undergo imaging brain scans at subsequent tumor evaluations, unless scans were clinically indicated. The same radiographic procedure used to assess disease sites at screening was used throughout the study (*e.g.*, the same contrast protocol for CT scans). Response was assessed by the investigator using RECIST v1.1 (*see* Eisenhauer *et al.* (2009) New response evaluation criteria in solid tumors: Revised RECIST guideline (Version 1.1). *Eur J Cancer*. 45: 228-47) and modified RECIST criteria. Modified RECIST criteria were derived from RECIST v1.1 (Eisenhauer *et al.*; Topalian *et al.* (2012) *N Engl J Med*. 366: 2443-54; and Wolchok *et al.* (2009) *Clin Can Res* 15: 7412-20) and immune-related response criteria (Wolchok *et al.*; Nishino *et al.* (2014) *J Immunother Can*. 2:17; and Nishino *et al.* (2013) *Clin Can Res*. 19:3936-43). Assessments were performed by the same evaluator, if possible, to ensure internal consistency across visits. Results were reviewed by the investigator before dosing at the next cycle.

[0388] Patients underwent tumor assessments every 6 weeks ($\pm$ 7 days) for 48 weeks following Cycle 1, Day 1 and then every 9 weeks ($\pm$ 7 days) thereafter, after the completion of the Week 48 tumor assessment, regardless of treatment delays, until radiographic disease progression per RECIST v1.1 (loss of clinical benefit for atezolizumab-treated patients who continue treatment beyond disease

progression according to RECIST v1.1 only), withdrawal of consent, death, or study termination by the Sponsor, whichever occurred first.

[0389] Patients who discontinued treatment for reasons other than radiographic disease progression per RECIST v1.1 (*e.g.*, toxicity, symptomatic deterioration) continued scheduled tumor assessments until radiographic disease progression per RECIST v1.1 (or loss of clinical benefit for atezolizumab-treated patients who had continued treatment with atezolizumab after radiographic disease progression according to RECIST v1.1), withdrawal of consent, death, or study termination by Sponsor, whichever occurred first.

[0390] Patients who started a new anti-cancer therapy in the absence of radiographic disease progression per RECIST v1.1 continued scheduled tumor assessments until radiographic disease progression per RECIST v1.1 (or loss of clinical benefit for atezolizumab-treated patients who had continued treatment with atezolizumab after radiographic disease progression according to RECIST v1.1), withdrawal of consent, death, or study termination by the Sponsor, whichever occurred first.

[0391] Tumor assessments for patients who were treated with atezolizumab who continued to experience clinical benefit, despite evidence of radiographic progression, was continued as per the schedule listed above.

Exploratory Analyses of Progression-Free Survival

[0392] ***Progression-Free Survival Rate at Landmark Time Points:*** The PFS rate, defined as the probability that a patient will be alive without disease progression after randomization (*e.g.*, at 6 months and at 1 year), was estimated using Kaplan-Meier methodology for each treatment arm, along with 95% CIs calculated using Greenwood's formula. The 95% CIs for the difference in PFS rates between the treatment arms was estimated using the normal approximation method, and standard errors were computed through use of the Greenwood method.

[0393] ***Non-Protocol-Specified Anti-Cancer Therapy:*** The impact of non-protocol-specified anti-cancer therapy on PFS was assessed depending on the number of patients who received non-protocol-specified anti-cancer therapy before a PFS event. If > 5% of patients received non-protocol-specified anti-cancer therapy before a PFS event in any treatment arm, a sensitivity analysis was performed for the comparisons between treatment arms in which patients who received non-protocol-specified anti-cancer therapy before a PFS event was censored at the last tumor assessment date before receipt of non-protocol-specified anti-cancer therapy.

[0394] ***Subgroup Analysis:*** To assess the consistency of the study results in subgroups defined by demographics (*e.g.*, age, sex, and race/ethnicity), baseline prognostic characteristics (*e.g.*, ECOG performance status, smoking status, and type of chemotherapy), the duration of PFS in these subgroups was examined. Summaries of PFS, including unstratified HRs estimated from Cox

proportional hazards models and Kaplan-Meier estimates of median PFS, were produced separately for each level of the categorical variables for the comparisons between treatment arms.

[0395] *Sensitivity Analyses:* Sensitivity analyses were performed to evaluate the potential impact of missing scheduled tumor assessments on the primary analysis of PFS, as determined by the investigator using a PFS event imputation rule. The following two imputation rules were considered: (1) If a patient missed two or more scheduled tumor assessments immediately prior to the date of the PFS event according to RECIST v1.1, the patient was censored at the last tumor assessment prior to the first of these missed visits. (2) If a patient missed two or more tumor assessments scheduled immediately prior to the date of the PFS event according to RECIST v1.1, the patient was counted as having progressed on the date of the first of these missing assessments. The imputation rule was applied to patients in both treatment arms.

[0396] The impact of loss to follow-up on OS will be assessed depending on the number of patients who are lost to follow-up. If > 5% of patients are lost to follow-up for OS in either treatment arm, a sensitivity analysis will be performed for the comparisons between treatment arms in which patients who are lost to follow-up will be considered as having died at the last date they were known to be alive.

Exploratory Analyses of Overall Survival

[0397] *Loss to Follow-Up:* The impact of loss to follow-up on OS was assessed depending on the number of patients who were lost to follow-up. If > 5% of patients were lost to follow-up for OS in either treatment arm, a sensitivity analysis was performed for the comparisons between treatment arms in which patients who were lost to follow-up were considered as having died at the last date they were known to be alive.

[0398] *Subgroup Analysis:* To assess the consistency of the study results in subgroups defined by demographics (*e.g.*, age, sex, and race/ethnicity), baseline prognostic characteristics (*e.g.*, ECOG performance status, smoking status, type of chemotherapy, presence of liver metastases at baseline), the duration of OS in these subgroups were examined. Summaries of survival, including unstratified HRs estimated from Cox proportional hazards models and Kaplan-Meier estimates of median survival time, were produced separately for each level of the categorical variables for the comparisons between treatment arms.

[0399] *Overall Survival Rate at 3-Year Landmark:* The OS rates at 3 years were estimated using Kaplan-Meier methodology for each treatment arm, along with 95% CIs calculated using the standard error derived from Greenwood's formula. The 95% CI for the difference in OS rates between the two treatment arms were estimated using the normal approximation method.

[0400] *Milestone Overall Survival Analysis:* To assess the effect of long-term survival and delayed clinical effects, a milestone OS analysis was conducted (Chen (2015) *J Natl Cancer Inst.*

107: djv156). The milestone OS was an OS endpoint with cross-sectional assessment at a pre-specified time point. The milestone OS analysis was performed using the same methods as those specified for the primary OS analysis

[0401] *Non-Protocol-Specified Anti-Cancer Therapy:* The impact of non-protocol-specified anti-cancer therapy on OS was assessed depending on the number of patients who receive such therapy. For example, the duration from initiation of non-protocol-specified anti-cancer therapy to death or censoring date may have been discounted in accordance with a range of possible effects on OS of subsequent non-protocol-specified anti-cancer therapy (*e.g.*, 10%, 20%, 30%).

[0402] *Exploratory Biomarker Analysis:* Exploratory biomarker analyses were performed in an effort to understand the association of these markers with study drug response, including efficacy and/or adverse events. The tumor biomarkers included but are not limited to PD-L1 and CD8, as defined by IHC, qRT-PCR, or other methods. Additional pharmacodynamic analyses were conducted as appropriate.

[0403] The Ventana anti-PD-L1 (SP142) rabbit monoclonal primary antibody immunohistochemistry (IHC) assay was used to determine programmed death-ligand 1 (PD-L1) IHC status.

[0404] *Device Description:* The Ventana anti-PD-L1 (SP142) rabbit monoclonal primary antibody is intended for use in the semi-quantitative immunohistochemical assessment of the PD-L1 protein in formalin-fixed paraffin-embedded non-small cell lung carcinoma (NSCLC) tissue stained on a Ventana BenchMark ULTRA automated slide stainer. It is indicated as an aid in the selection of NSCLC patients with locally advanced or metastatic disease who might benefit from treatment with atezolizumab.

[0405] The Ventana anti-PD-L1 (SP142) rabbit monoclonal primary antibody is a pre-dilute, ready-to-use antibody product optimized for use with the Ventana Medical Systems OptiView DAB IHC Detection Kit and the OptiView Amplification Kit on Ventana Medical Systems automated BenchMark ULTRA platforms. One 5-mL dispenser of anti-PD-L1 (SP142) rabbit monoclonal primary antibody contains approximately 36 µg of rabbit monoclonal antibody directed against the PD-L1 protein and contains sufficient reagent for 50 tests. The reagents and the IHC procedure are optimized for use on the BenchMark ULTRA automated slide stainer, utilizing Ventana System Software (VSS).

[0406] *Scoring System:* PD-L1 staining with anti-PD-L1 (SP142) rabbit monoclonal primary antibody in NSCLC can be observed in both tumor cells and tumor-infiltrating immune cells using the Ventana anti-PD-L1 (SP142) rabbit monoclonal primary antibody.

Results

[0407] The results of the study are presented in *Table 10* below:

Table 10: Summary of Primary Efficacy Endpoints

INV-PFS	ITT	ARM A (n=292)	ARM B (n=286)
	Median (months)	7.6 (+2.4m)	5.2
	Stratified HR (95% CI)	0.596 (0.494, 0.719)	
	Stratified Log-rank p ($\alpha = 0.002$)	<.0001	
OS	Median (months)	18.1 (+4.5m)	13.6
	Stratified HR (95% CI)	0.813 (0.644, 1.025)	
	Stratified Log-rank p ($\alpha = 0.0406$)	0.0797	

[0408] *Table 10* shows that the study demonstrated a statistically significant and clinically meaningful improvement in the investigator-assessed progression-free survival (PFS) in the ITT population. Additionally, the study demonstrated a numerical improvement in overall survival (OS).

[0409] Patients treated with atezolizumab + pemextrexed + carboplatin or cisplatin demonstrated extended progression-free survival as compared to patients treated with pemetrexed + carboplatin or cisplatin. See **FIG. 2**. The 6 month PFS of patients receiving atezolizumab + pemextrexed + carboplatin or cisplatin was 59.14% vs. 40.93% in patients receiving pemetrexed + carboplatin or cisplatin. The 12 month PFS of patients receiving atezolizumab + pemetrexed + carboplatin or cisplatin was 33.71% vs. 16.97% in patients receiving pemetrexed + carboplatin or cisplatin. Patients treated with atezolizumab + pemextrexed + carboplatin or cisplatin demonstrated numerically improved overall survival as compared to patients treated with pemetrexed + carboplatin or cisplatin. See **FIG. 3** (NE = not evaluated). The 6 month OS of patients receiving atezolizumab + pemextrexed + carboplatin or cisplatin was 59.61% vs. 55.39% in patients receiving Placebo + pemextrexed + carboplatin or cisplatin. The 12 month OS of patients receiving atezolizumab + pemextrexed + carboplatin or cisplatin was 59.6% vs. 55.4% in patients receiving Placebo + pemextrexed + carboplatin or cisplatin.

[0410] In addition, the confirmed overall response rate (ORR) in patients treated with atezolizumab + pemextrexed + carboplatin or cisplatin was 47%, whereas the confirmed ORR in patients treated with pemetrexed + carboplatin or cisplatin was 32% (CR: 1.7% in Arm A vs. 0.7 in Arm B; CR/PR: 46.9% in Arm A vs. 32.2% in Arm B). See **FIG. 4**. (CR = complete response; CR/PR = complete response/partial response; SD = stable disease; PD = progressive disease.) Unconfirmed ORR was also improved in Arm A. As shown in *Table 11* below, the median confirmed duration of response (DOR) in patients receiving atezolizumab + pemetrexed + carboplatin or cisplatin (*i.e.*, Arm A) was 10.1 months, whereas the median confirmed DOR in patients receiving

pemetrexed + carboplatin or cisplatin (*i.e.*, Arm B) was 7.2 months. DOR was assessed according to RECIST v1.1 criteria. Unconfirmed DOR was also improved in Arm A. 42% of the patients in Arm A demonstrated ongoing response, as compared to 30% of the patients in Arm B.

Table 11: Median Confirmed Duration of Response in Treatment Arms A and B

DOR	Arms	
	ARM B	ARM A
Median DOR (95% CI), mo	7.2 (5.7, 9.0)	10.1 (7.2, 13.3)
No. of ongoing response, n (%)	28 (30.4%)	58 (42.3%)

[0411] The PFS benefit was observed across all subgroups analyzed. *See FIG. 5A*. Numerical OS improvement was also observed. *See FIG. 6*. Consistent results were shown across clinical subgroups.

[0412] The safety profile of atezolizumab + pemetrexed + carboplatin or cisplatin was consistent with the known risks of the individual treatment components. No new safety signals were identified. Key safety parameters are consistent with the findings from other first-line NSCLC studies involving atezolizumab in combination with platinum based chemotherapy.

[0413] This study demonstrated that initial (first-line) treatment with the combination of atezolizumab + pemetrexed + carboplatin or cisplatin reduced the risk of disease worsening or death (PFS) compared to chemotherapy (*i.e.*, pemetrexed + carboplatin or cisplatin) alone. Numerical improvement in overall survival was also seen in patients treated with atezolizumab + pemetrexed + carboplatin or cisplatin as compared to patients treated with chemotherapy (*i.e.*, pemetrexed + carboplatin or cisplatin) alone.

Example 2: Efficacy of atezolizumab in combination with carboplatin + pemetrexed or cisplatin + pemetrexed as first-line treatment in key subgroups of patients with Stage IV non-squamous non-small cell lung cancer (NSCLC)

[0414] Exploratory efficacy analyses examining PFS and interim OS in clinically relevant patient subgroups (*e.g.*, race, age, smoking history, and liver metastasis at baseline) were conducted based on the results described in *Example 1*.

[0415] 578 patients were enrolled. The median follow-up was 14.8 mo. Baseline characteristics were mostly balanced between treatment arms. *See Table 12* and **FIG. 5B** for PFS and interim OS data in key subgroups.

Table 12: PFS and Interim OS data in Key Subgroups

Population	APP	PP	
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	n	Median PFS (95% CI), mo	n	Median PFS (95% CI), mo	HR (95% CI); P value
ITT	292	7.6 (6.6, 8.5)	286	5.2 (4.3, 5.6)	0.596 (0.494, 0.719); P < 0.0001 ^a
Non-Asian	221	6.9	221	5.0	0.65 (0.53, 0.81)
Asian	71	10.2	65	5.3	0.42 (0.028, 0.63)
< 65 y	153	6.9	167	4.4	0.63 (0.49, 0.80)
≥ 65 y	139	8.4	119	5.6	0.55 (0.42, 0.73)
Never smokers	37	8.6	30	5.5	0.49 (0.28, 0.87)
Former/current smokers	255	7.5	256	5.1	0.61 (0.50, 0.74)
No liver metastases	255	8.4	250	5.5	0.56 (0.46, 0.69)
Liver metastases	37	4.4	36	4.0	0.77 (0.47, 1.25)
Population	APP		PP		
	n	Median OS (95% CI), mo	n	Median OS (95% CI), mo	HR (95% CI); P value
ITT	292	18.1 (13.0, NE)	286	13.6 (11.4, 15.5)	0.813 (0.644, 1.025) P = 0.0797 ^a
Non-Asian	221	13.0	221	11.0	0.82 (0.64, 1.06)
Asian	71	NE	65	NE	0.68 (0.37, 1.24)
< 65 y	153	18.8	167	14.2	0.89 (0.62, 1.21)
≥ 65 y	139	18.1	119	12.8	0.71 (0.50, 1.01)
Never smokers	37	18.1	30	13.3	0.65 (0.32, 1.30)
Former/current smokers	255	18.8	256	13.6	0.83 (0.65, 1.06)

No liver metastases	255	19.9	250	14.2	0.76 (0.59, 0.98)
Liver metastases	37	10.1	36	6.9	0.99 (0.57, 1.70)
^a Stratified.					

[0416] The addition of Atezolizumab to carboplatin or cisplatin + pemetrexed resulted in numerical improvement in PFS and OS in most key clinical subgroups. Survival benefit appeared more pronounced in Asian patients, older patients, and never smokers.

Example 3: Exploratory Analysis: Progression-Free Survival by PD-L1 Status in Biomarker-Evaluable Patients from Example 1

[0417] PD-L1 expression levels on tumor-infiltrating immune cells (IC) and on tumor cells (TC) in baseline tissue samples obtained from biomarker-evaluable patients (*i.e.*, from **Example 1**) were analyzed. Tumor cells were scored as TC0, TC1, TC2, or TC3, and tumor-infiltrating immune cells were scored as IC0, IC1, IC2, and IC3.

[0418] The overall response rate (ORR) and progression free survival rate (PFS) for patients scored as TC3 or IC3 (*i.e.*, “PD-L1 High”); TC1, TC2, IC1, or IC2 (or “PD-L1 low”); and TC0 or IC0 (*i.e.*, “PD-L1 negative”) were analyzed for each treatment arm. The results of these analyses are shown in **FIGs. 7A, 7B, and 7C**.

[0419] As shown in **FIG. 7A**, “PD-L1 High” patients who received atezolizumab + pemetrexed + carboplatin or cisplatin demonstrated an ORR of 72%. By contrast, the ORR of “PD-L1 High” patients who were treated with pemetrexed + carboplatin was 55%. The median PFS of “PD-L1 High” patients who received atezolizumab + pemetrexed + carboplatin or cisplatin was 10.8 months, whereas the median PFS of “PD-L1 High” patients in the control arm was 6.5 months. The 12-month PFS among “PD-L1 High” patients in the treatment arm was 46%, whereas the 12-month PFS among “PD-L1 High” patients in the control arm was 25%.

[0420] There were no significant differences in ORR or median PFS in “PD-L1 Low” patients in the treatment arm as compared to the control arm. The 12-month PFS among “PD-L1 Low” patients in the treatment arm was 27%, and the 12-month PFS among “PD-L1 Low” patients in the control arm was 20%. *See FIG. 7B*.

[0421] **FIG. 7C** shows that “PD-L1 negative” patients who received atezolizumab + pemetrexed + carboplatin or cisplatin demonstrated an ORR of 44%. By contrast, the ORR of “PD-L1 negative” patients who were treated with pemetrexed + carboplatin was 27%. The median PFS of “PD-L1 Negative” patients who received atezolizumab + pemetrexed + carboplatin or cisplatin was 8.5 months, whereas the median PFS of “PD-L1 Negative” patients in the control arm was 4.9 months. The 12-month PFS among “PD-L1 Negative” patients in the treatment arm was 35%, whereas the 12-month PFS among “PD-L1 Negative” patients in the control arm was 8%. The

median duration of response (DOR) among “PD-L1 Negative” patients in the treatment arm was 10.1 months, whereas the median duration of response (DOR) among “PD-L1 Negative” patients in the control arm was 4.2 months. Although the present disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the present disclosure. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

CLAIMS

1. A method of treating an individual having lung cancer, comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an antimetabolite, and a platinum agent, wherein the treatment extends progression free survival (PFS) of the individual.
2. The method of claim 1, wherein the treatment extends overall survival (OS) of the individual.
3. A method of treating an individual having lung cancer, comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an antimetabolite, and a platinum agent, wherein the treatment extends overall survival (OS) of the individual.
4. The method of claim 1, wherein the treatment extends the PFS of the individual by at least about 6 months.
5. The method of claim 2 or 3, wherein the treatment extends the OS of the individual is extended by at least about 15 months.
6. The method of any one of claims 1-5, wherein the anti-PD-L1 antibody comprises:
 - (a) a heavy chain variable region (V_H) that comprises an HVR-H1 comprising an amino acid sequence of GFTFSDSWIH (SEQ ID NO: 1), an HVR-2 comprising an amino acid sequence of AWISPYGGSTYYADSVKG (SEQ ID NO: 2), and HVR-3 comprising an amino acid RHWPGGFDY (SEQ ID NO: 3), and
 - (b) a light chain variable region (V_L) that comprises an HVR-L1 comprising an amino acid sequence of RASQDVSTAVA (SEQ ID NO: 4), an HVR-L2 comprising an amino acid sequence of SASFLYS (SEQ ID NO: 5), and an HVR-L3 comprising an amino acid sequence of QQYLYHPAT (SEQ ID NO: 6).
7. The method of any one of claims 1-6, wherein the anti-PD-L1 antibody comprises a heavy chain variable region (V_H) comprising an amino acid sequence of SEQ ID NO: 7 and a light chain variable region (V_L) comprising an amino acid sequence of SEQ ID NO: 8.
8. The method of any one of claims 1-7, wherein the anti-PD-L1 antibody is atezolizumab.

9. The method of any one of claims 1-8, wherein the antimetabolite is pemetrexed, 5-fluorouracil, 6-mercaptopurine, capecitabine, cytarabine, floxuridine, fludarabine, hydroxycarbamide, or methotrexate.
10. The method of claim 9, wherein the antimetabolite is pemetrexed.
11. The method of claim any one of claims 1-10, wherein the platinum agent is carboplatin.
12. The method of any one of claims 1-10, wherein the platinum agent is cisplatin.
13. The method of any one of claims 1-11, wherein the anti-PD-L1 antibody is administered at a dose of 1200 mg, wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve $AUC = 6 \text{ mg/ml/min}$, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m^2 .
14. The method of any one of claims 1-10 and 12, wherein the anti-PD-L1 antibody is administered at a dose of 1200 mg, wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m^2 , and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m^2 .
15. The method of any one of claims 1-11 and 13, wherein the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m^2 Day 1, and wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve $AUC = 6 \text{ mg/ml/min}$ on Day 1 of each 21-day cycle for Cycles 1-4.
16. The method of any one of claims 1-10, 12, and 14, wherein the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m^2 Day 1, and wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m^2 on Day 1 of each 21-day cycle for Cycles 1-4.
17. The method of any one of claims 15-16, wherein the anti-PD-L1 antibody, antimetabolite, and the platinum agent are administered sequentially on Day 1 of Cycles 1-4.

18. The method of claim 17, wherein the anti-PD-L1 antibody is administered prior to the antimetabolite, and wherein the antimetabolite is administered prior to the platinum agent on Day 1 of Cycles 1-4.
19. The method of any one of claims 15-18, wherein the anti-PD-L1 antibody and the antimetabolite are further administered following Cycle 4, wherein the anti-PD-L1 antibody is atezolizumab and is administered at a dose of 1200 mg on Day 1, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4.
20. The method of claim 19, wherein the anti-PD-L1 antibody and the antimetabolite are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4.
21. The method of claim 20, wherein the anti-PD-L1 antibody is administered prior to the antimetabolite on Day 1 of after Cycle 4.
22. The method of any one of claims 1-11 and 13, wherein the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is carboplatin and is administered at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle for Cycles 1-6.
23. The method of any one of claims 1-10, 12, and 14, wherein the anti-PD-L1 antibody, the antimetabolite, and the platinum agent are administered in four 21-day Cycles, and wherein the anti-PD-L1 antibody is atezolizumab and administered at a dose of 1200 mg on Day 1, wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² Day 1, and wherein the platinum agent is cisplatin and is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6.
24. The method of claim 22 or 23, wherein the anti-PD-L1 antibody, antimetabolite, and the platinum agent are administered sequentially on Day 1 of Cycles 1-6.
25. The method of claim 24, wherein the anti-PD-L1 antibody is administered prior to the antimetabolite, and wherein the antimetabolite is administered prior to the platinum agent on Day 1 of Cycles 1-6.

26. The method of any one of claims 22-25, wherein the anti-PD-L1 antibody and the antimetabolite are further administered following Cycle 6, wherein the anti-PD-L1 antibody is atezolizumab and is administered at a dose of 1200 mg on Day 1, and wherein the antimetabolite is pemetrexed and is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6.
27. The method of claim 26, wherein the anti-PD-L1 antibody and the antimetabolite are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6.
28. The method of claim 27, wherein the anti-PD-L1 antibody is administered prior to the antimetabolite on Day 1 of each 21-day cycle for every cycle after Cycle 6.
29. The method of any one of claims 1-28, wherein the anti-PD-L1 antibody, the platinum agent, and the antimetabolite inhibitor are each administered intravenously.
30. The method of any one of claims 1-29, wherein the lung cancer is non-small cell lung cancer (NSCLC).
31. The method of claim 30, wherein the NSCLC is Stage IV non-squamous NSCLC.
32. The method of claim 31, wherein the individual is treatment-naïve for Stage IV non-squamous NSCLC.
33. The method of claim 31, wherein the individual is chemotherapy-naïve for Stage IV non-squamous NSCLC.
34. The method of any one of claims 1-33, wherein the individual is Asian.
35. The method of any one of claims 1-34, wherein the individual is at least 65 years old.
36. The method of any one of claims 1-35, wherein the individual is a never smoker.
37. The method of any one of claims 1-36, wherein the individual is PD-L1 high.
38. The method of any one of claims 1-36, wherein the individual is PD-L1 negative.

39. A method of treating an individual having Stage IV non-squamous non-small cell lung cancer (NSCLC), comprising administering to the individual an effective amount of atezolizumab, pemetrexed, and carboplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min, wherein the treatment extends progression free survival (PFS).

40. The method of claim 39, wherein treatment extends the overall survival (OS) of the individual.

41. The method of claim 39 or 40, wherein atezolizumab, pemetrexed, and carboplatin are administered in four 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 4; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-4, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4, and carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min on Day 1 of each 21-day cycle of Cycles 1-4, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 4 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4.

42. The method of claim 41, wherein atezolizumab, pemetrexed, and carboplatin are administered sequentially on Day 1 of Cycles 1-4.

43. The method of claim 42, wherein atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to carboplatin on Day 1 of Cycles 1-4.

44. The method of any one of claims 41-43, wherein atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4.

45. The method of claim 44, wherein atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 4.

46. The method of claim 39 or 40, wherein atezolizumab, pemetrexed, and carboplatin are administered in six 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 6, wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-6, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each

21-day cycle for Cycles 1-6, and carboplatin is administered at a dose sufficient to achieve $AUC = 6$ mg/ml/min on Day 1 of each 21-day cycle of Cycles 1-6, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 6 and pemetrexed is further administered at a dose of 500 mg/m^2 on Day 1 of each 21-day cycle for every cycle after Cycle 6.

47. The method of claim 46, wherein atezolizumab, pemetrexed, and carboplatin are administered sequentially on Day 1 of Cycles 1-6.

48. The method of claim 47, wherein atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to carboplatin on Day 1 of Cycles 1-6.

49. The method of any one of claims 46-48, wherein atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6.

50. The method of claim 49, wherein atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 6.

51. The method of any one of claims 39-50, wherein the atezolizumab, the pemetrexed, and the carboplatin are each administered intravenously.

52. The method of any one or claims 39-41, wherein treatment extends the PFS of the individual by at least about 6 months.

53. The method of any one or claims 39-52, wherein treatment extends the OS of the individual by at least about 15 months.

54. A method of treating an individual having Stage IV non-squamous non-small cell lung cancer (NSCLC), comprising administering to the individual an effective amount of atezolizumab, pemetrexed, and cisplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m^2 , and the cisplatin is administered at a dose of 75 mg/m^2 , wherein the treatment extends progression free survival (PFS) of the individual.

55. The method of claim 54, wherein treatment extends the overall survival (OS) of the individual.

56. The method of claim 54 or 55, wherein atezolizumab, pemetrexed, and cisplatin are administered in four 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 4; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-4, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-4, and cisplatin is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle of Cycles 1-4, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 4 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 4.

57. The method of claim 56, wherein atezolizumab, pemetrexed, and cisplatin are administered sequentially on Day 1 of Cycles 1-4.

58. The method of claim 57, wherein atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to cisplatin on Day 1 of Cycles 1-4.

59. The method of any one of claims 56-58, wherein atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 4.

60. The method of claim 59, wherein atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 4.

61. The method of claim 54 or 55, wherein atezolizumab, pemetrexed, and cisplatin are administered in six 21-day Cycles, and atezolizumab and pemetrexed are further administered in 21-day cycles following Cycle 6; wherein atezolizumab is administered at a dose of 1200 mg on Day 1 of each 21-day cycle of Cycles 1-6, pemetrexed is administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for Cycles 1-6, and cisplatin is administered at a dose of 75 mg/m² on Day 1 of each 21-day cycle of Cycles 1-6, and wherein atezolizumab is further administered at a dose of 1200 mg on Day 1 of each 21-day cycle for every cycle after Cycle 6 and pemetrexed is further administered at a dose of 500 mg/m² on Day 1 of each 21-day cycle for every cycle after Cycle 6.

62. The method of claim 61, wherein atezolizumab, pemetrexed, and cisplatin are administered sequentially on Day 1 of Cycles 1-6.

63. The method of claim 62, wherein atezolizumab is administered prior to pemetrexed, and wherein pemetrexed is administered prior to cisplatin on Day 1 of Cycles 1-6.

64. The method of any one of claims 61-63, wherein atezolizumab and pemetrexed are administered sequentially on Day 1 of each 21-day cycle for every cycle after Cycle 6.
65. The method of claim 64, wherein atezolizumab is administered prior to pemetrexed on Day 1 of each 21-day cycle for every cycle after Cycle 6.
66. The method of any one of claims 54-65, wherein the atezolizumab, the pemetrexed, and the cisplatin are each administered intravenously.
67. The method of any one or claims 54-66, wherein treatment extends the PFS of the individual by at least about 6 months.
68. The method of any one or claims 54-67, wherein treatment extends the OS of the individual by at least about 15 months.
69. The method of any one of claims 39-68, wherein the individual is treatment-naïve for Stage IV non-squamous NSCLC.
70. The method of any one of claims 39-69, wherein the individual is chemotherapy-naïve for Stage IV non-squamous NSCLC.
71. The method of any one of claims 39-70, wherein the individual is Asian.
72. The method of any one of claims 39-71, wherein the individual is at least 65 years old.
73. The method of any one of claims 39-72, wherein the individual is a never smoker.
74. The method of any one of claims 39-73, wherein the individual is PD-L1 high.
75. The method of any one of claims 39-74, wherein the individual is PD-L1 negative.
76. The method of any one of claims 1-75, wherein the individual is human.
77. A kit comprising an anti-PD-L1 antibody for use in combination with an antimetabolite and platinum agent for treating an individual having lung cancer according to a method of any one of claims 1-38 and 76.

78. A kit comprising atezolizumab for use in combination with pemetrexed and carboplatin for treating an individual having lung cancer according to a method of any one of claims 39-53 and 69-76.

79. A kit comprising atezolizumab for use in combination with pemetrexed and cisplatin for treating an individual having lung cancer according to a method of any one of claims 54-76.

80. An anti-PD-L1 antibody for use in a method of treating lung cancer in an individual, the method comprising administering to the individual an effective amount of an anti-PD-L1 antibody, an antimetabolite, and a platinum agent, wherein the treatment extends progression free survival (PFS) and/or overall survival (OS) of the individual.

81. The anti-PD-L1 antibody of claim 80, for use in a method is according to any one of claims 1-38 and 76.

82. A composition comprising atezolizumab for use in a method of treating Stage IV non-squamous non-small cell lung cancer (NSCLC), the method comprising administering to the individual an effective amount of atezolizumab, pemetrexed and carboplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the carboplatin is administered at a dose sufficient to achieve AUC = 6 mg/ml/min, and wherein the treatment extends progression free survival (PFS) and/or overall survival (OS) of the individual.

83. The composition of claim 82, for use in a method according to any one of claims 39-53 and 69-76.

84. A composition comprising atezolizumab for use in a method of treating Stage IV non-squamous non-small cell lung cancer (NSCLC), the method comprising administering to the individual an effective amount of atezolizumab, pemetrexed and cisplatin, wherein the atezolizumab is administered at a dose of 1200 mg, the pemetrexed is administered at a dose of 500 mg/m², and the cisplatin is administered at a dose of 75 mg/m², and wherein the treatment extends progression free survival (PFS) and/or overall survival (OS) of the individual.

85. The composition of claim 84, for use in a method according to any one of claims 54-76.

FIG. 1

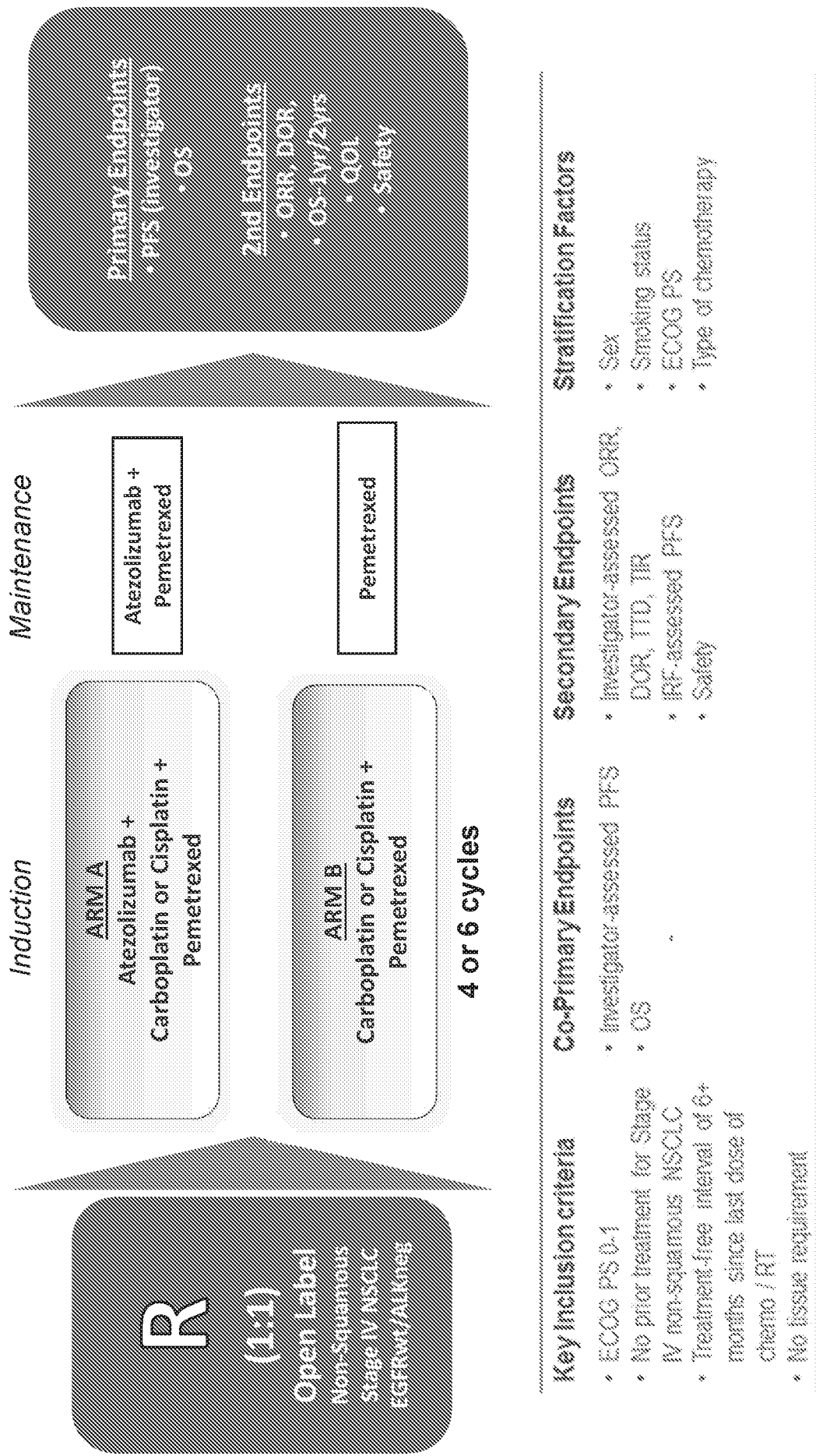
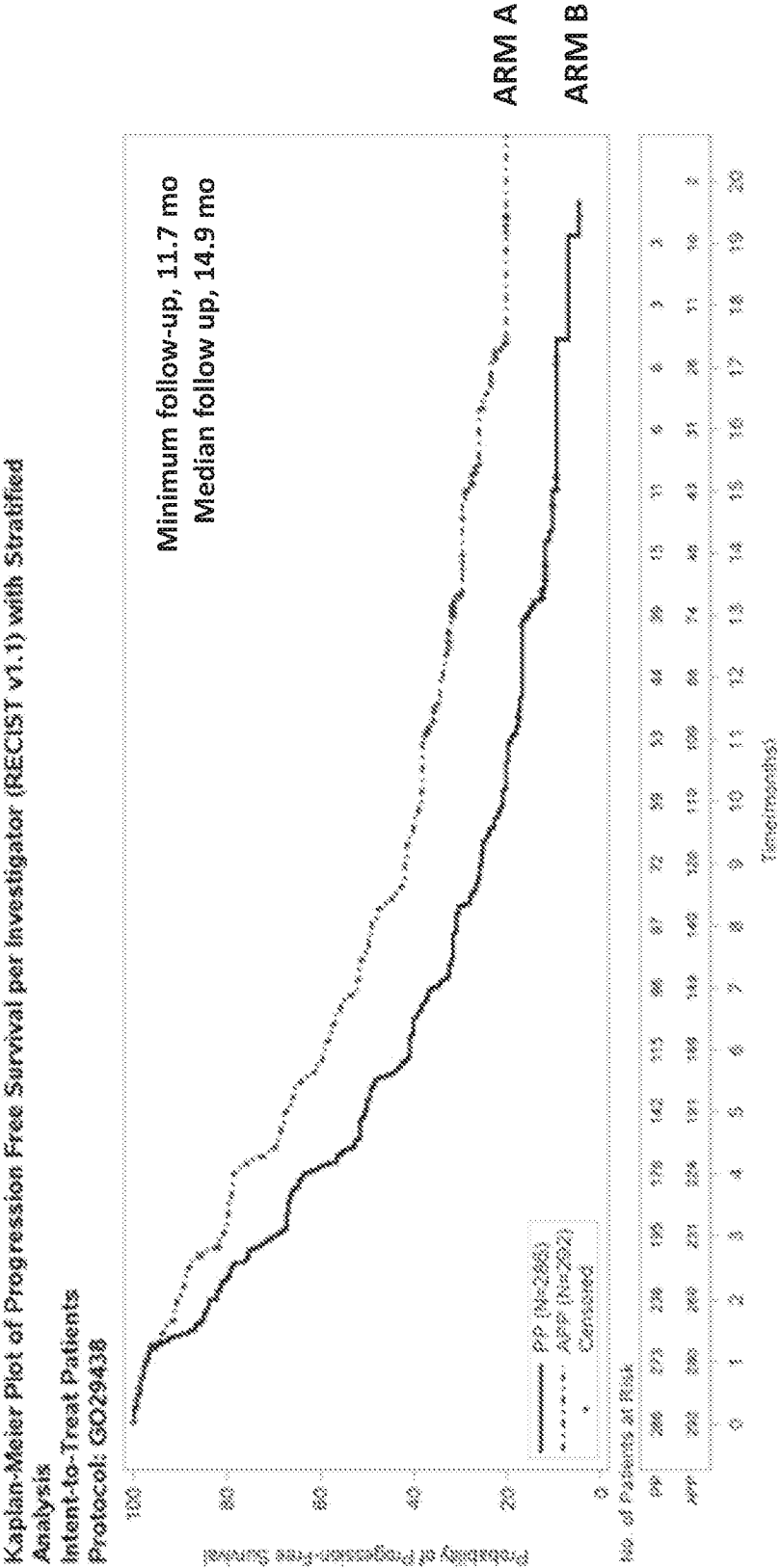


FIG. 2



	ARM B	ARM A
Median (months)	5.2	7.6
Stratified HR (95% CI)	0.596 (0.494, 0.719)	
Stratified Log-rank p-Value	<.0001	

Landmark	ARM B	ARM A
6 month	40.93%	59.14%
12 month	16.97%	33.71%

FIG. 3

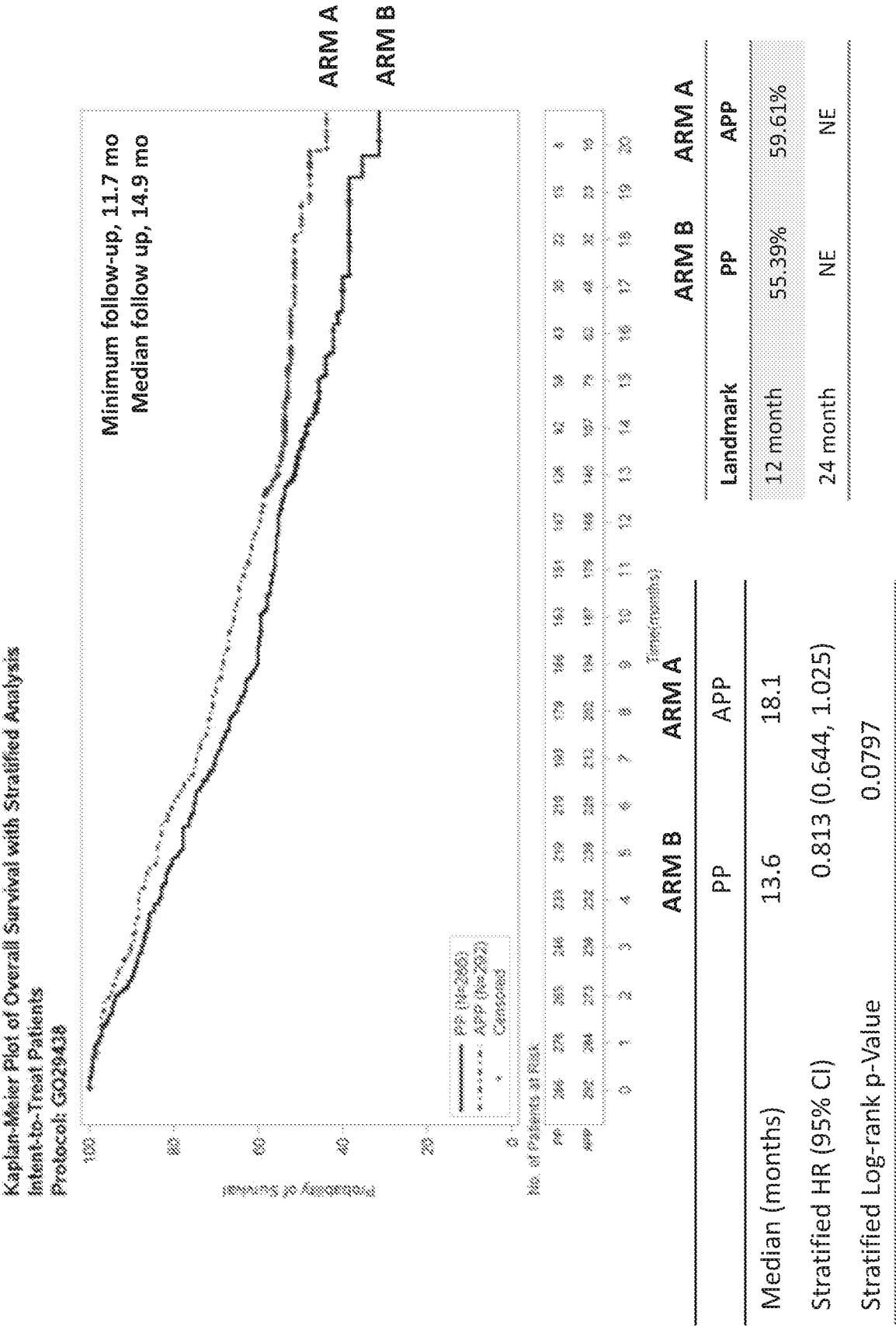


FIG. 4

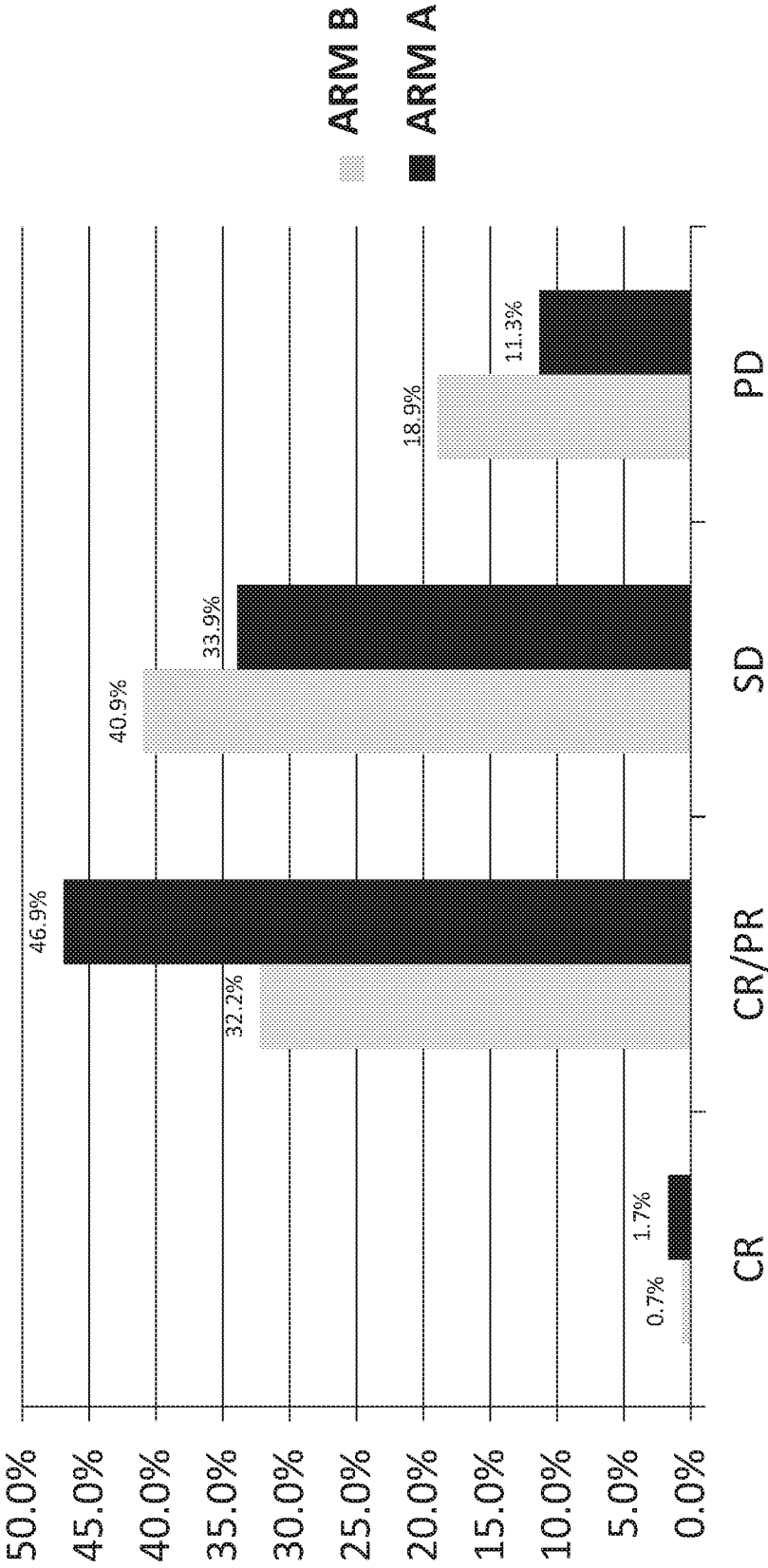


FIG. 5A

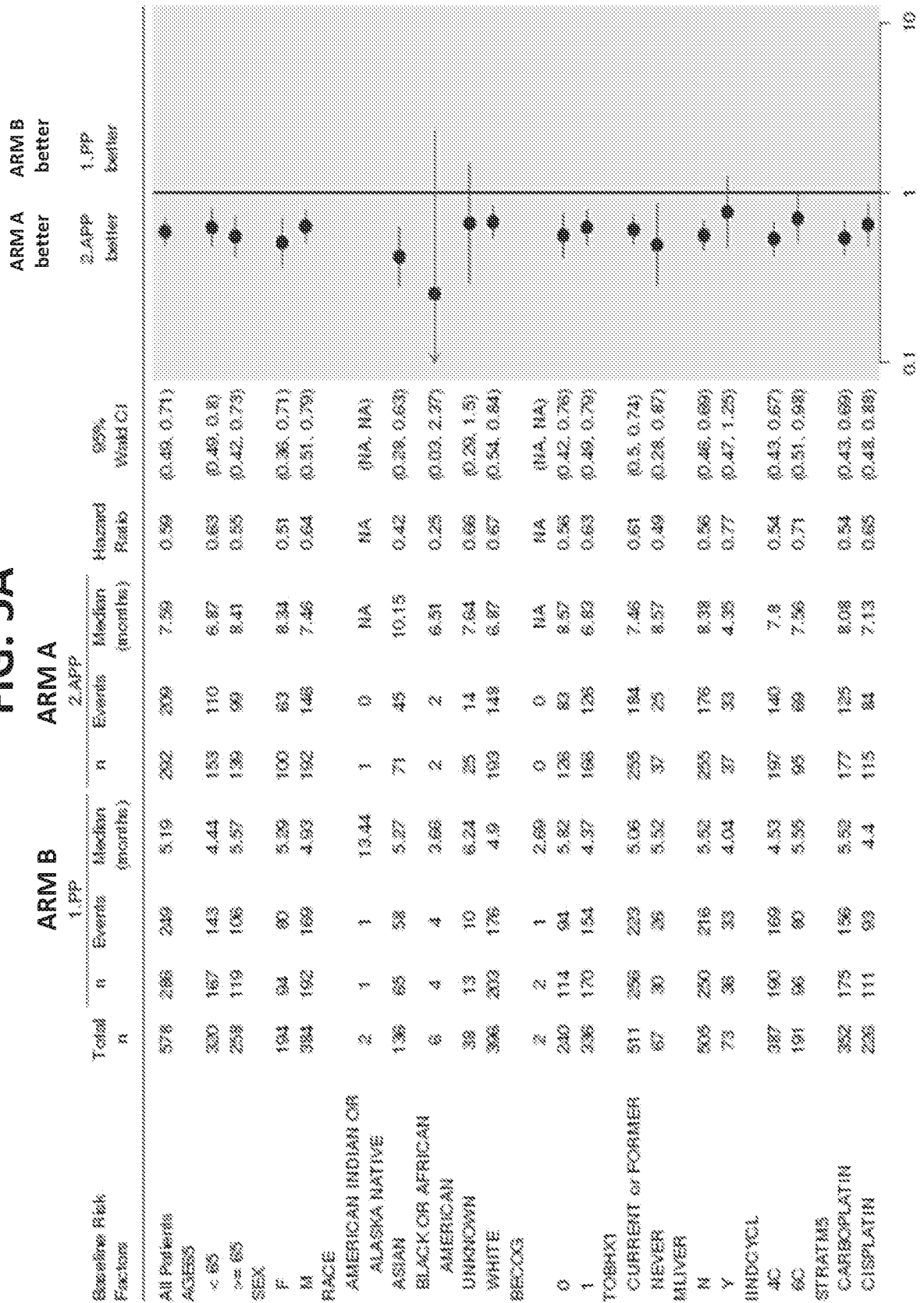
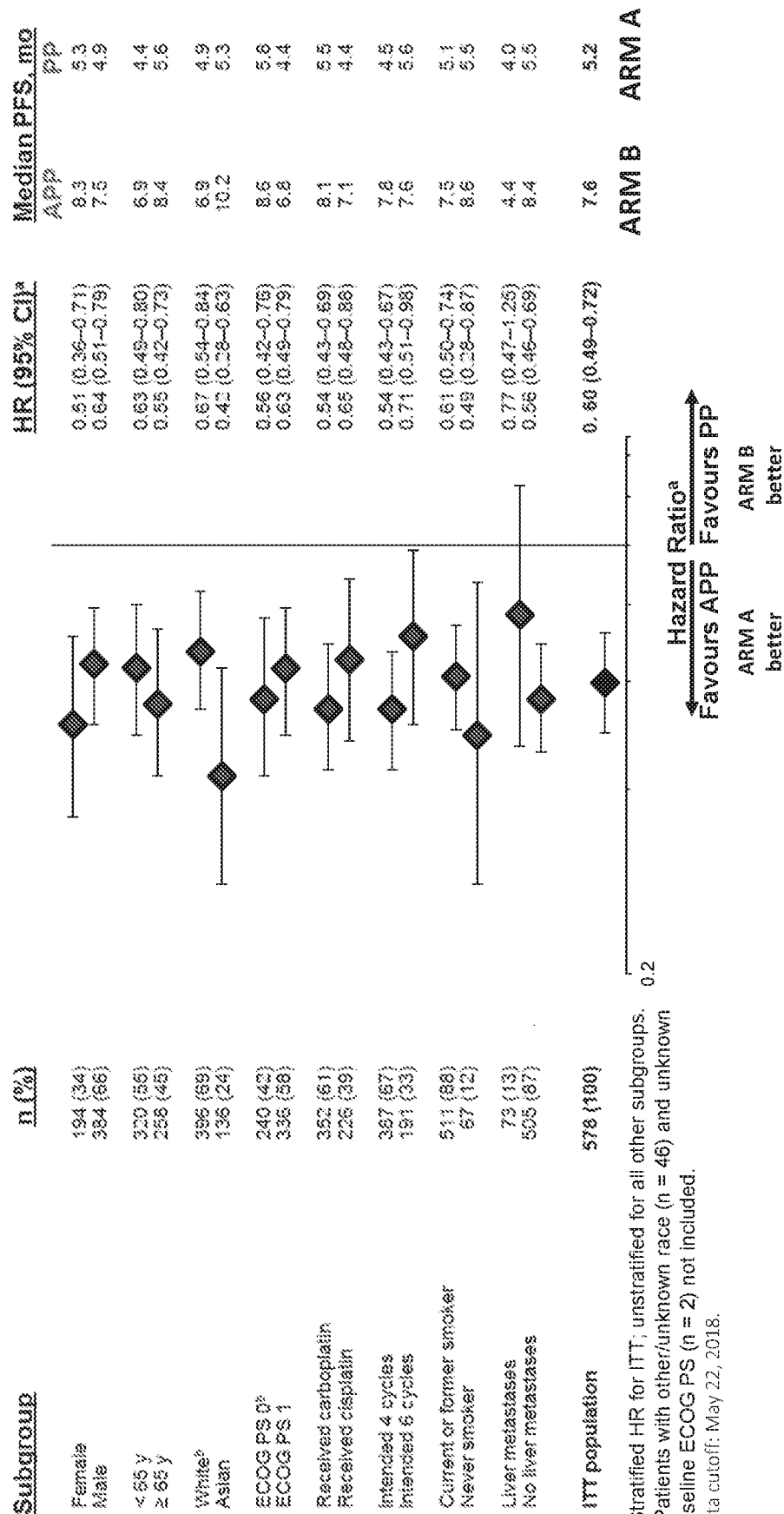


FIG. 5B

^a Stratified HR for ITT, unstratified for all other subgroups.^b Patients with other/unknown race (n = 46) and unknown baseline ECOG PS (n = 2) not included.

Data cutoff: May 22, 2018.

FIG. 6

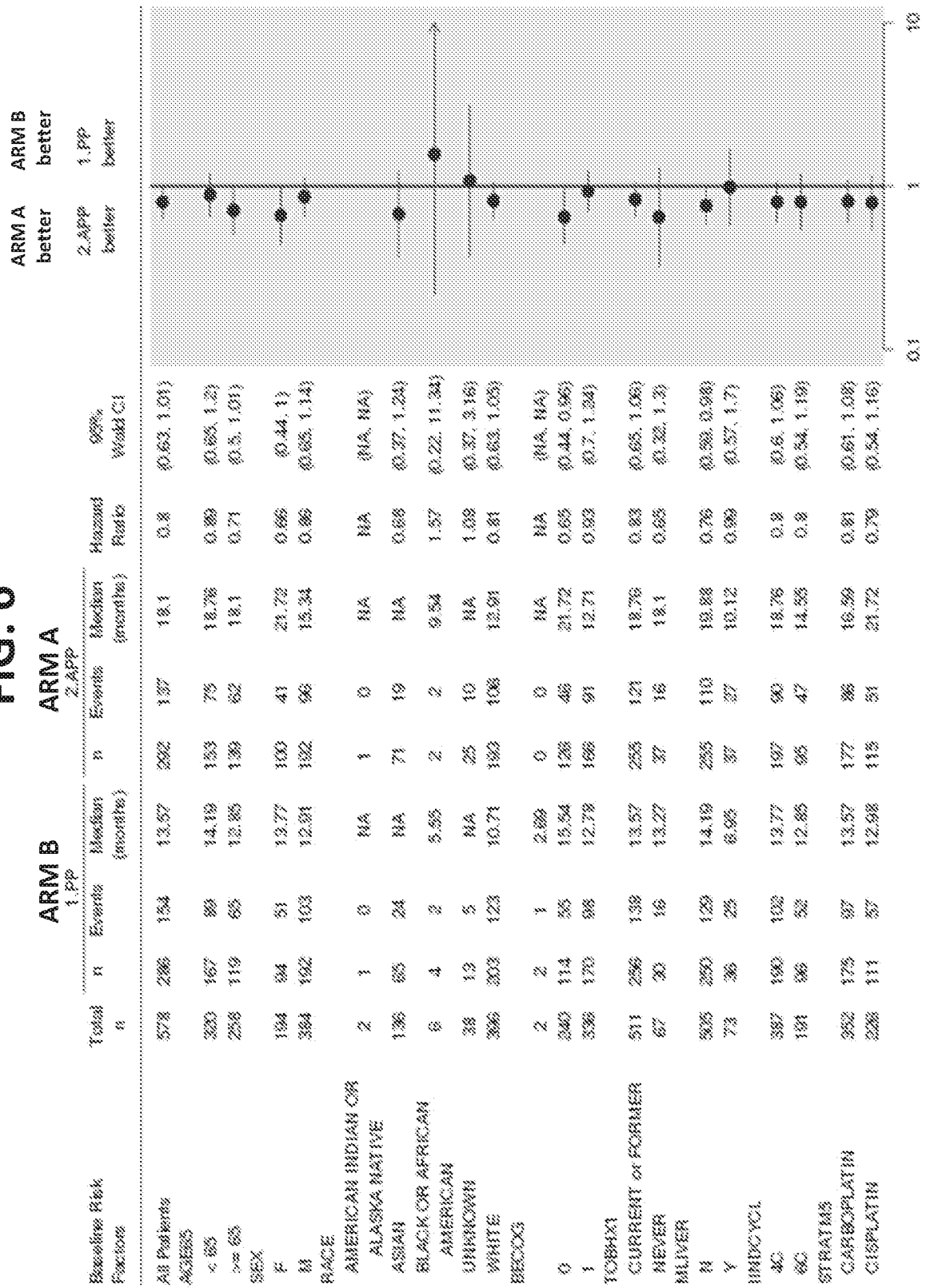


FIG. 7A

PD-L1 High
TC3 or IC3

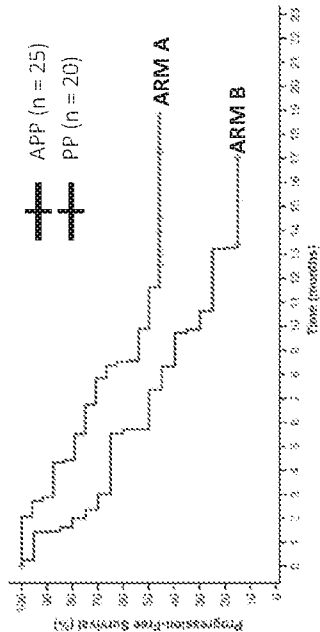


FIG. 7B

PD-L1 Low
TC1/2 or IC1/2

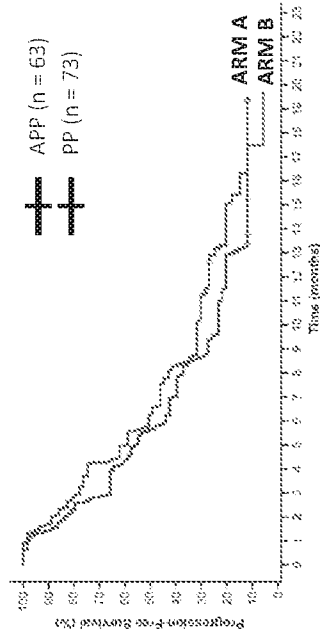
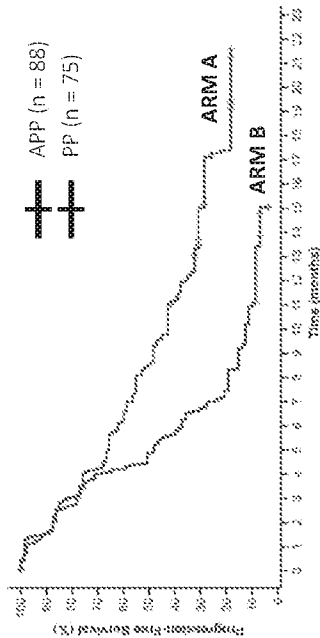


FIG. 7C

PD-L1 Negative
TC0 and IC0



	APP	PP	APP	PP	APP	PP
ORR, %	72%	55%	38%	38%	44%	27%
CR PR, %	0 72%	5% 50%	2% 37%	0 38%	2% 42%	0 27%
Median DOR, mo	NE	7.2	7.2	7.2	10.1	4.2
12-month PFS	46%	25%	27%	20%	35%	8%
Median PFS, mo	10.8	6.5	6.2	5.7	8.5	4.9
HR ^a (95% CI)	0.46 (0.22, 0.96)		0.80 (0.56, 1.16)		0.45 (0.31, 0.64)	

Overall HR 0.57 (0.45, 0.73) in biomarker-evaluable patients (60% of ITT). Unstratified HR. Data cutoff: May 22, 2018.

APP = Arm A; PP = Arm B