

specifically binding to PD-1 have been reported (see, *e.g.*, Patnaik A. *et al.* (2015) “Phase I Study of Pembrolizumab (MK-3475; Anti-PD-1 Monoclonal Antibody) in Patients with Advanced Solid Tumors,” Clin Cancer Res; 21(19):4286-4293; US 7,488,802; US 7,521,051; US 7,595,048; US 8,008,449; US 8,354,509; US 8,735,553; US 8,779,105; US 8,900,587; US 9,084,776; US 9,815,897; US 10,577,422; WO 2014/194302; and WO 2015/035606; WO 2004/056875; WO 2006/121168; WO 2008/156712; WO 2012/135408; WO 2012/145493; WO 2013/014668; WO 2014/179664; WO 2014/194302; WO 2015/112800; and WO 2019/246110).

[0007] Combination therapy using separate intravenous doses of the anti-CTLA-4 antibody ipilimumab and the anti-PD-1 antibody nivolumab with chemotherapy have recently been approved for the treatment of for certain patients with metastatic or recurrent non-small cell lung cancer (NSCLC). However, combination therapy was accompanied by increased frequency and severity of treatment-related adverse events (TRAEs). Fifty-five percent of patients receiving the combination of ipilimumab and nivolumab experienced severe TRAEs, a significant increase compared to 16% for nivolumab alone and 27% for ipilimumab alone (Larkin, J., et al., 2015. “*Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma*,” N. Engl. J. Med.). Beyond the potential medical consequences of severe TRAEs for cancer patients, TRAEs often necessitate discontinuation of treatment, limiting the therapeutic benefit in these populations.

[0008] Bispecific molecules binding to both PD-1 and CTLA-4 allow for great flexibility in the design and engineering in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. The use of PD-1 x CTLA-4 bispecific molecules in the treatment of cancer has been proposed and PD-1 x CTLA-4 bispecific molecules have been described for example in WO 2014/209804; WO 2017/218707; WO 2017/193032; WO 2019/094637; and US 2019/0185569. In particular, tetravalent PD-1 X CTLA-4 bispecific diabodies and trivalent PD-1 x CTLA-4 binding molecules having exemplary activity are described in WO 2017/106061.

SUMMARY ~~OF THE INVENTION~~

[0009] Provided are dosing regimens for the administration of PD-1 x CTLA-4 bispecific molecules for the treatment of cancer and other diseases and conditions that can minimize undesirable side effects. The present ~~invention~~disclosure also pertains in part to methods of using such

PD-1 x CTLA-4 bispecific molecules to stimulate immune cells. The inventiondisclosure concerns in part the use of such regimens for the administration of tetravalent PD-1 x CTLA-4 bispecific diabodies that comprise two binding sites for PD-1 and two binding sites for CTLA-4. The inventiondisclosure is directed in part to the use of such bispecific molecules. The inventiondisclosure is also directed in part to the use of pharmaceutical compositions and pharmaceutical kits that contain such molecules, which facilitate the use of such dosing regimens in the treatment of cancer or to stimulate immune cells.

[0010] In detail, the inventiondisclosure provides a method of treating cancer comprising administering a PD-1 x CTLA-4 bispecific molecule to a subject in need thereof, wherein the PD-1 x CTLA-4 bispecific molecule comprises at least one PD-1 Binding Domain and at least one CTLA-4 Binding Domain, and wherein the method comprises administering the PD-1 x CTLA-4 bispecific molecule to the subject at a dose of from about 3 mg/kg to about 10 mg/kg once every 3 weeks. In one aspect, the present disclosure refers to use of a PD-1 x CTLA-4 bispecific molecule in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein said PD-1 x CTLA-4 bispecific molecule comprises a PD-1 Binding Domain and a CTLA-4 Binding Domain, and wherein said PD-1 x CTLA-4 bispecific molecule is to be administered to a subject at a dose of about 3 mg/kg to about 10 mg/kg once every 3 weeks. The inventiondisclosure further provides an embodimentexample of such method, wherein the PD-1 x CTLA-4 bispecific molecule is administered to the subject at a dose of from about 3 mg/kg to about 10 mg/kg once every 3 weeks during an induction period.

[0011] The inventiondisclosure further provides a method of stimulating immune cells comprising administering a PD-1 x CTLA-4 bispecific molecule to a subject in need thereof, wherein the PD-1 x CTLA-4 bispecific molecule comprises at least one PD-1 Binding Domain and at least one CTLA-4 Binding Domain, and wherein the method comprises administering the PD-1 x CTLA-4 bispecific molecule to the subject at a dose of from about 3 mg/kg to about 10 mg/kg once every 3 weeks. In one aspect, the present disclosure refers to use of a PD-1 x CTLA-4 bispecific molecule in the manufacture of a medicament for stimulating immune cells in a subject in need thereof, wherein said PD-1 x CTLA-4 bispecific molecule comprises a PD-1 Binding Domain and a CTLA-4 Binding Domain, and wherein said PD-1 x CTLA-4 bispecific molecule is to be administered to a subject at a dose of about 3 mg/kg to about 10 mg/kg once every 3 weeks. The inventiondisclosure further provides an embodimentexample of such method, wherein the PD-1 x CTLA-4 bispecific molecule is administered to the subject at a dose of from about 3 mg/kg to about 10 mg/kg once every 3 weeks during an induction period. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the immune cells are T cells.

[0012] The inventiondisclosure further provides the embodimentexample of such methods, wherein:

- (I) the PD-1 Binding Domain comprises a Light Chain Variable Domain (VL_{PD-1}) that comprises the CDRL1, CDRL2 and CDRL3 of **SEQ ID NO:1**, and a Heavy Chain Variable Domain (VH_{PD-1}) that comprises the PD-1-specific CDRH1, CDRH2 and CDRH3 of **SEQ ID NO:5**; and
- (II) the CTLA-4 Binding Domain comprises a Light Chain Variable Domain (VL_{CTLA-4}) that comprises the CDRL1, CDRL2 and CDRL3 of **SEQ ID NO:9**,

and a Heavy Chain Variable Domain (VH_{CTLA-4}) that comprises the CTLA-4-specific CDRH1, CDRH2 and CDRH3 of **SEQ ID NO:13**.

[0013] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule comprises:

- (I) two of the PD-1 binding domains; and
- (II) two of the CTLA-4 binding domains.

[0014] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 binding domain comprises the VL Domain of **SEQ ID NO:1** and the VH Domain of **SEQ ID NO:5**.

[0015] The inventiondisclosure further provides the embodimentexample of such methods, wherein the CTLA-4 binding domain comprises the VL Domain of **SEQ ID NO:9** and the VH Domain of **SEQ ID NO:13**.

[0016] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule comprises an Fc Region. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the Fc Region is of the IgG1, IgG2, IgG3, or IgG4 isotype.

[0017] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule further comprises a Hinge Domain.

[0018] The inventiondisclosure further provides the embodimentexample of such methods, wherein the Fc Region and the Hinge Domain are both of the IgG4 isotype, and wherein the Hinge Domain comprises a stabilizing mutation.

[0019] The inventiondisclosure further provides the embodimentexample of such methods, wherein the Fc Region is a variant Fc Region that comprises:

- (a) one or more amino acid modifications that reduces the affinity of the variant Fc Region for an FcγR; and/or
- (b) one or more amino acid modifications that enhances the serum half-life of the variant Fc Region.

[0020] The inventiondisclosure further provides the embodimentexample of such methods, wherein the modifications that reduces the affinity of the variant Fc Region for an FcγR comprise the

substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

[0021] The inventiondisclosure further provides the embodimentexample of such methods, wherein the modifications that enhances the serum half-life of the variant Fc Region comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

[0022] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is a diabody comprising one polypeptide chain that comprises the amino acid sequence of **SEQ ID NO:40** and a second polypeptide chain that comprises the amino acid sequence of **SEQ ID NO:41**.

[0023] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is a diabody comprising two polypeptide chains each comprising the amino acid sequence of **SEQ ID NO:40** and two polypeptide chains each comprising the amino acid sequence of **SEQ ID NO:41**.

[0024] Also provided is an embodimentexample of such methods, in which the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 3 mg/kg and 8 mg/kg.

[0025] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 6 mg/kg and about 10 mg/kg.

[0026] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 6 mg/kg.

[0027] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 7 mg/kg.

[0028] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 8 mg/kg.

[0029] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 9 mg/kg.

[0030] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, further comprising administering the PD-1 x CTLA-4 bispecific molecule to the subject at a dose of from about 3 mg/kg to about 10 mg/kg once every 6 weeks during a maintenance period, wherein the maintenance period follows the induction period.

[0031] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the induction period has a duration of up to about 24 weeks.

[0032] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the maintenance period has a duration of at least 6 weeks. The [inventiondisclosure](#) particularly provides the [embodimentexample](#) of such methods, wherein the maintenance period has a duration of at least 84 weeks.

[0033] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 3 mg/kg and about 8 mg/kg during the induction period.

[0034] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 6 mg/kg and about 10 mg/kg during the induction period.

[0035] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 3 mg/kg during the induction period.

[0036] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 4 mg/kg during the induction period.

[0037] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 5 mg/kg during the induction period.

[0038] The [inventiondisclosure](#) further provides the [embodimentexample](#) of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 6 mg/kg during the induction period.

[0039] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 6.5 mg/kg during the induction period.

[0040] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 7 mg/kg during the induction period.

[0041] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 7.5 mg/kg during the induction period.

[0042] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 8 mg/kg during the induction period.

[0043] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 8.5 mg/kg during the induction period.

[0044] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 9 mg/kg during the induction period.

[0045] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 9.5 mg/kg during the induction period.

[0046] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 10 mg/kg during the induction period.

[0047] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 3 mg/kg and 8 mg/kg during the maintenance period.

[0048] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of between about 6 mg/kg and about 10 mg/kg during the maintenance period.

[0049] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 3 mg/kg during the maintenance period.

[0050] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 4 mg/kg during the maintenance period.

[0051] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 5 mg/kg during the maintenance period.

[0052] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 6 mg/kg during the maintenance period.

[0053] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 6.5 mg/kg during the maintenance period.

[0054] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 7 mg/kg during the maintenance period.

[0055] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 7.5 mg/kg during the maintenance period.

[0056] The inventiondisclosure further provides the embodimentexample of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 8 mg/kg during the maintenance period.

[0057] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 8.5 mg/kg during the maintenance period.

[0058] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 9 mg/kg during the maintenance period.

[0059] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 9.5 mg/kg during the maintenance period.

[0060] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered at a dose of about 10 mg/kg during the maintenance period.

[0061] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the dose of PD-1 x CTLA-4 bispecific molecule administered in the maintenance period is the same as the dose administered in the induction period.

[0062] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the dose of PD-1 x CTLA-4 bispecific molecule administered in the maintenance period is different than the dose administered in the induction period.

[0063] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the PD-1 x CTLA-4 bispecific molecule is administered by intravenous (IV) infusion.

[0064] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the IV infusion is over a period of between about 30 minutes to about 60 minutes.

[0065] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the cancer is selected from the group consisting of: an adrenal gland cancer, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, an anal cancer, a bile duct cancer, a bladder cancer, a bone cancer, a brain cancer, a brain and spinal cord cancer, a breast cancer, a HER2+ breast cancer, a triple negative breast cancer (TNBC), a carotid body tumors, a cervical cancer, an HPV-related cervical cancer, a cervical squamous cell carcinoma, a chondrosarcoma, a chordoma, a clear cell carcinoma, a colon cancer, a colorectal cancer

(CRC), a microsatellite instability-high colorectal cancer (MSI-H CRC), a microsatellite-stable colorectal cancer (non-microsatellite-instability-high colorectal cancer, non-MSI-H CRC), a desmoplastic small round cell tumor, an endometrial cancer, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a fallopian tube carcinoma, a fibrogenesis imperfecta ossium, a fibrous dysplasia of the bone, a gallbladder or bile duct cancer, a gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a glioblastoma, a head and neck cancer, an HPV-related head and neck cancer, a hematological malignancy, a hepatocellular carcinoma, an islet cell tumor, a Kaposi's Sarcoma, a kidney cancer, a leukemia, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a non-small-cell lung cancer (NSCLC), a medulloblastoma, a melanoma, a meningioma, Merkel cell carcinoma, a mesothelioma pharyngeal cancer, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumor, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a pheochromocytoma, a pituitary tumor, a prostate cancer, a metastatic castration resistant prostate cancer (mCRPC), a posterior uveal melanoma, a renal cancer, a renal cell carcinoma (RCC), a rhabdoid tumor, a rhabdomyosarcoma, a sarcoma, a skin cancer, a small round blue cell tumor of childhood (including neuroblastoma and rhabdomyosarcoma), , a soft-tissue sarcoma, a pleomorphic undifferentiated sarcoma, a dedifferentiated liposarcoma, a synovial sarcoma, a myxofibrosarcoma, a squamous cell cancer, a squamous cell cancer of the head and neck (SCCHN), a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid cancer, a thyroid metastatic cancer, and a uterine cancer.

[0066] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is selected from the group consisting of: cervical cancer, HPV-related cervical cancer, cervical squamous cell carcinoma, CRC, MSI-H CRC, non-MSI-H CRC, head and neck cancer, HPV-related head and neck cancer, lung cancer, melanoma, NSCLC, prostate cancer, renal cancer, RCC, soft-tissue sarcoma, a pleomorphic undifferentiated sarcoma, a dedifferentiated liposarcoma, a synovial sarcoma, a myxofibrosarcoma, squamous cell cancer, and SCCHN.

[0067] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is cervical cancer. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the cervical cancer is cervical squamous cell carcinoma.

[0068] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is CRC. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the CRC is non-MSI-H CRC or is MSI-H CRC.

[0069] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is lung cancer. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the lung cancer is NSCLC.

[0070] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is melanoma. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the melanoma is cutaneous melanoma.

[0071] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is prostate cancer. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the prostate cancer is metastatic castration-resistant prostate cancer (mCRPC).

[0072] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is renal cancer. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the renal cancer is RCC.

[0073] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is soft tissue sarcoma. The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the cancer is pleomorphic undifferentiated sarcoma, dedifferentiated liposarcoma, synovial sarcoma, or myxofibrosarcoma.

[0074] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is squamous cell cancer.

[0075] The inventiondisclosure further provides the embodimentexample of such methods, wherein the cancer is head and neck cancer.

[0076] The inventiondisclosure particularly provides the embodimentexample of such methods, wherein the squamous cell cancer or the head and neck cancer is SCCHN.

[0077] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, further comprising administering a therapeutically or prophylactically effective amount of one or more additional therapeutic agents or chemotherapeutic agents.

[0078] The ~~invention~~disclosure further provides the ~~embodiment~~example of such methods, wherein the subject in need thereof is a human.

[0079] The ~~invention~~disclosure provides a pharmaceutical kit comprising:
(a) a container comprising a PD-1 x CTLA-4 bispecific molecule; and
(b) an instructional material,
wherein the instructional material instructs that said PD-1 x CTLA-4 bispecific molecule is to be used according to the method of any of the above ~~embodiment~~examples. In one aspect, the present disclosure refers to a pharmaceutical kit comprising:
(a) a container comprising a PD-1 x CTLA-4 bispecific molecule; and
(b) an instructional material,
wherein the instructional material instructs that said PD-1 x CTLA-4 bispecific molecule is to be used according to the use as disclosed herein.

[0080] The ~~invention~~disclosure provides an ~~embodiment~~example for the use of such pharmaceutical kit according to such methods for the treatment of cancer.

[0081] The ~~invention~~disclosure provides an ~~embodiment~~example for the use of such pharmaceutical kit according to such methods for stimulating immune cells.

[0081a] In one aspect, the present disclosure refers to the pharmaceutical kit as disclosed herein for use in the treatment of cancer or for stimulating immune cells.

[0082] The ~~invention~~disclosure provides an ~~embodiment~~example for the use of such PD-1 x CTLA-4 bispecific molecule according to such methods for the treatment of cancer.

[0083] The ~~invention~~disclosure provides an ~~embodiment~~example for the use of such PD-1 x CTLA-4 bispecific molecule according to such methods for stimulating immune cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0084] **Figure 1** provides a schematic showing representative covalently bonded tetravalent diabody, such as a PD-1 x CTLA-4 bispecific diabody, having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair has an E-coil Heterodimer-Promoting Domain and the other polypeptide of each pair has a K-coil Heterodimer-Promoting Domain. As shown, a cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain. One polypeptide of each pair possesses a linker comprising a cysteine (which linker may comprise all or a portion of a hinge region) and CH2 and/or CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The VL and VH Domains recognize different