

COMPOSITIONS AND METHODS COMPRISING ANTIBODIES THAT BIND TO COVALENT PEPTIDE CONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional patent application no. 63/154,627,
5 filed February 26, 2021, and U.S. provisional patent application no. 63/253,499, filed
October 7, 2021, the entire disclosures of each of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under grant nos. CA194864,
CA267362 and CA049152 awarded by the National Institutes of Health. The government has
10 certain rights in the invention.

SEQUENCE LISTING

The instant application contains a Sequence Listing, which has been submitted
electronically in ASCII format and is hereby incorporated by reference in its entirety. Said
ASCII copy, created on February 24, 2022, is named 58636_00482_SL.txt, and is 212,998
15 bytes in size.

BACKGROUND

There is an ongoing and unmet need for agents that can bind to targets that include
drugs that are covalently bound to proteins or peptides. In particular, there is a need to
improve the efficacy of targeted therapy and also to increase tumor immunogenicity and the
20 efficacy of immune therapy against cancer driven by intracellular oncogenes. The disclosure
is pertinent to these needs.

SUMMARY

The present disclosure provides compositions and methods that include binding
partners that bind with specificity to target sites on proteins or peptides that comprise a
25 covalently attached molecule. It is believed that this is the first disclosure of binding partners
with this binding function. The disclosure illustrates this approach using binding partners in
the form of numerous antibodies and antibody derivatives that specifically bind to proteins
and peptides that have been covalently modified by attachment of a molecule, wherein the
molecules are illustrated by a variety of drugs. Further, the disclosure demonstrates binding
30 partners that bind with specificity to peptides that have been covalently modified by
attachment of a small molecule drug are specific for the described covalently modified
peptides when presented in the context of a human leukocyte antigen (HLA), wherein HLA is a

representative example of a major histocompatibility complex (MHC). Thus, binding partners that are specific for peptide-drug conjugates in an HLA complex are demonstrated. The disclosure includes polynucleotides encoding the described binding partners and cells that are modified to express the binding partners. The disclosure includes diagnostic, prophylactic and therapeutic approaches using the binding partners.

In one aspect, the present disclosure relates to a composition comprising a binding partner or a polynucleotide encoding the binding partner, wherein the binding partner binds to a peptide conjugate in complex with a major histocompatibility complex (MHC), wherein the peptide conjugate comprises a peptide covalently linked to a small molecule, and wherein a dissociation constant (Kd) of the binding partner to the peptide conjugate in complex with the MHC is lower than a Kd of the binding partner to the peptide conjugate not in complex with the MHC.

In another aspect, the present disclosure relates to the composition as disclosed herein for use in therapy. In another aspect, the present disclosure relates to use of the composition as disclosed herein in the manufacture of a medicament for the treatment of cancer in a subject in need thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Graphs depicting results of phage enzyme-linked immunosorbent assay (ELISA) of phage-displayed antibody clones. Binding to KRAS^{G12C}-GDP and KRAS^{G12C}-GDP-ARS-1620 conjugate was determined. For each clone, the bars in the graph are, from left to right, Buffer, G12C-GDP-ARS, and G12C-GDP.

Figure 2. Graph showing 12C-ARS Fab59 binding to KRAS^{G12C} in GTPγS- or GDP-bound nucleotide state with or without ARS-1620 was characterized using the bead binding assay.

Figure 3. Graphs showing that 12C-ARS-Fab59 specifically binds KRAS^{G12C}-GDP conjugated to ARS-1620.

Figure 4. Shown in (A) is data obtained using 12C-ARS-Fab59 to measure ARS-1620/KRAS^{G12C} adducts by pull-down assays from lysates prepared from cell lines. Shown in (B) are immunoblots of whole cell lysates and 12C-ARS Fab-pull-downs (PD) from RAS-less MEFs reconstituted with the indicated *KRAS* mutants (4A) and KCP (*Kras*^{G12C}; *Tp53*^{R172H}; Pdx-Cre) mouse pancreas cancer cells (4B), treated in the presence or absence of ARS-1620. Shown in (C) are whole cell lysates and 12C-ARS Fab pull-downs (PD) from H358 and MIAPaCa-2 cells, treated as indicated. Shown in (D) is ARS-adduct formation in samples from C, quantified by LC/MS-MS assay. ARS-1620 and SHP099 concentrations were 10 μM in all panels.

Figure 5. Data showing that 12C-ARS-Fab59 can be used to measure the engagement of ARS-1620 to mutant KRAS by pull-down assay with lysates prepared from animal tissues. A, B, anti-pan RAS and anti-ERK2 (loading control) immunoblots of lysates and 12C-ARS Fab pull-downs (PD) from *LSL-KRAS*^{G12C}-*Tp53*^{R270H} (A) and *LSL-KRAS*^{G12C} (B) tumors after 3 days of oral gavage with ARS-1620 (200 mg/kg/d) alone or with the SHP2 inhibitor SHP099 (75 mg/kg/d) are shown.

Figure 6. Binding of antibody clones to AMG510 conjugated to the KRAS(G12C) peptide and the poly-Ser peptide. For each antibody clone, the bars are from left to right are

What is claimed is:

1. A composition comprising a binding partner or a polynucleotide encoding the binding partner, wherein the binding partner binds to a peptide conjugate
5 in complex with a major histocompatibility complex (MHC), wherein the peptide conjugate comprises a peptide covalently linked to a small molecule, and wherein a dissociation constant (K_d) of the binding partner to the peptide conjugate in complex with the MHC is lower than a K_d of the binding partner to the peptide conjugate not in complex with the MHC.
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2. The composition of claim 1, wherein the K_d of the binding partner to the peptide conjugate in complex with the MHC is at most 20 nM.
3. The composition of claim 1 or 2, wherein the peptide conjugate comprises a Cys
15 residue that is covalently linked to the small molecule.
4. The composition of any one of claims 1-3, wherein the peptide comprises a mutation.
5. The composition of any one of claims 1-4, wherein the small molecule is selected
20 from the group consisting of AMG510, 2E07, 6H05, SML-8-73-1, MRTX849, JNJ74699157, LY3499446, ARS-853, ARS-1620, MRTX1275, and derivatives thereof.
6. The composition of any one of claims 1-5, wherein the peptide is from a RAS protein that is selected from the group consisting of KRAS, HRAS and NRAS; Bruton's tyrosine
25 kinase (BTK); an epidermal growth factor receptor (EGFR) family member that is selected from EGFR (ERBB1), HER2/NEU (ERBB2), HER3 (ERBB3), and HER4 (ERBB4); MET (HGFR); any fibroblast growth factor receptor (FGFR); a cyclin-dependent kinase (CDK); Acetylcholine Esterase (ACHE); p90 ribosomal S6 kinase (RSK); TP53, IDH1, GNAS, FBXW7, CTNNB1, DNMT3A, a cathepsin that is selected from the group consisting of
30 cathepsin B, C, F, H, K, L, O, S, V, W and X; a caspase; a pancreatic lipase; or METAP2.
7. The composition of claim 6, wherein the peptide is from KRAS and wherein the peptide comprises a KRAS^{G12C} mutation.

8. The composition of any one of claims 1-7, wherein the peptide comprises the amino acid sequence of SEQ ID NO:1 or SEQ ID NO:2.
9. The composition of any one of claims 1-8, wherein the peptide conjugate comprises a peptide from KRAS having a G12C mutation covalently linked to AMG510.
10. The composition of any one of claims 1-9, wherein the MHC is HLA-A*03:01 or HLA-A*11:01.
11. The composition of claim 6, wherein the peptide is from EGFR or BTK, wherein the small molecule is osimertinib or ibrutinib, and wherein the peptide conjugate is in complex with HLA-A*02:01 or HLA-A*01:01.
12. The composition of any one of claims 1-11, wherein the binding partner is an intact antibody, an antigen-binding (Fab) fragment, an Fab' fragment, an (Fab')₂ fragment, an Fd, an Fv, a dAb, a single domain fragment or single monomeric variable antibody domain, a single-chain Diabody (scDb), a single-chain variable fragment (scFv), a bi-specific T-cell engager (BiTE), bispecific killer cell engager (BiKE), CrossMab, a tri-specific binding partner, or a chimeric antigen receptor (CAR).
13. The composition of claim 12, wherein the binding partner is a bi-specific T-cell engager comprising a first antigen-binding domain that binds to the peptide conjugate in complex with the MHC and a second antigen-binding domain that binds to CD3ε of a T cell.
14. The composition of claim 12 or 13, wherein the binding partner comprises (i) a heavy chain having a heavy chain (HC) complementarity determining region (CDR) 1 of an amino acid sequence of DYSIH, a HC CDR2 of an amino acid sequence of SISSSSGSTSYADSVKG, and a HC CDR3 of an amino acid sequence of X1X2X3X4X5X6MDY, where X1 is G, X2 is selected from the group consisting of A, C, E, G, H, K, L, M, N, P, Q, R, S, T, W and Y, X3 is selected from the group consisting of G, P, R, T and W, X4 is selected from the group consisting of A, D, E, F, G, H, I, K, M, N, Q, R, S, T, W and Y, X5 is selected from the group consisting of A, P and V, X6 is selected from the group consisting of A, C, G, K, L, M, Q, R, S, T and Y, wherein one, two, or three of X1X2X3X4X5X6 are mutated relative to a parental sequence of SEQ ID NO:155; (ii) a light

chain having a light chain (LC) CDR1 of an amino acid sequence of RASQSVSSAVA, a LC CDR2 of an amino acid sequence of SASSLYS, and a LC CDR3 of an amino acid sequence of QQX1X2X3X4X5X6X7X8T, where X1 is selected from the group consisting of A, I, L, P, S, T and V, X2 is selected from the group consisting of K, R, S and T, X3 is F or Y, X4 is
5 selected from the group consisting of I, R, T and V, X5 is selected from the group consisting of A, E, H, K, L, M, N, Q, R, S, T and Y, X6 is K or R, X7 is selected from the group consisting of A, C, D, E, G, H, K, L, M, N, P, Q, R, S, T, V and W, and X8 is selected from the group consisting of I, L and V, wherein one, two, or three of X1X2X3X4X5X6X7X8 are mutated relative to a parental sequence of SEQ ID NO:153.

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15. The composition of claim 14, wherein the binding partner comprises a heavy chain having at least 80% sequence identity to SEQ ID NO: 4, and a light chain having at least 80% sequence identity to SEQ ID NO: 3.

15 16. The composition of any one of claims 12-15, wherein the binding partner comprises (i) a heavy chain having a heavy chain (HC) complementarity determining region (CDR) 1 of an amino acid sequence of DYSIH, a HC CDR2 of an amino acid sequence of SSSSSGSTSYADSVKG, and a HC CDR3 of an amino acid sequence of GGWYPAMDY; (ii) a light chain having a light chain (LC) CDR1 of an amino acid sequence of
20 RASQSVSSAVA, a LC CDR2 of an amino acid sequence of SASSLYS, and a LC CDR3 of an amino acid sequence of QQISYVKKLIT.

17. The composition of claim 16, wherein the binding partner comprises a heavy chain having at least 80% sequence identity to SEQ ID NO: 4, and a light chain having at least 80%
25 sequence identity to SEQ ID NO: 3.

18. The composition of any one of claims 1-17, wherein the binding partner does not bind, or binds with a lower affinity, to the peptide that is not covalently linked to a small molecule.

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19. The composition of any one of claims 1-18 for use in therapy.

20. Use of the composition of any one of claims 1-18 in the manufacture of a medicament for the treatment of cancer in a subject in need thereof.

21. The use of claim 20, wherein the cancer is renal cell carcinoma, breast cancer, prostate cancer, pancreatic cancer, lung cancer, liver cancer, ovarian cancer, cervical cancer, colon cancer, esophageal cancer, glioma, glioblastoma or another brain cancer, stomach
5 cancer, bladder cancer, testicular cancer, head and neck cancer, melanoma or another skin cancer, a sarcoma, or a blood cancer.
22. The use of claim 20 or 21, wherein the subject has been administered the small molecule.