MULTIVALENT MULTISPECIFIC CONSTRUCTS FOR TARGETED PAYLOAD DELIVERY

TECHNICAL FIELD

The disclosure relates, *inter alia*, to multivalent multispecific constructs conjugated to therapeutic payloads, and to use of the constructs in treating diseases or disorders.

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. U.S. 63/562,832, filed March 8, 2024, the entire contents of which are hereby incorporated by reference in its entirety.

DESCRIPTION OF THE XML FILE SUBMITTED ELECTRONICALLY

The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML file, created on February 18, 2025, is named "SDL-001PC_136782-5001_Sequence_Listing" and is 25,313 bytes in size.

BACKGROUND

Current methods for targeted delivery of payloads to specific cells are not efficient enough to realize the full benefit of such approaches. Utilizing current formats, such as IgG antibody drug conjugates, targeted payload delivery is highly target expression dependent. In the setting of cancer, few tumor associated antigens (TAAs) are expressed at high enough levels for efficient specific payload delivery to achieve optimal biologic effects and clinical outcomes. For example, antibody drug conjugates against Her2+ overexpression such as fam-trastuzumab dxd, can lead to high response rates approximating 80% with prolonged duration of response approximating 2 years in high antigen expressing cancer patients (<25% of breast cancer, <2% of lung cancer). However, no other TAAs are expressed at levels approaching Her2. Other approved ADCs targeting lower expressed TAAs such as Trop2, have not shown benefits approaching that for Her2. In general, the current methods are limited by payload internalization efficiency, which are then impacted by low efficacy and by toxicity. Given this, there is a great need for targeted payload delivery methods that are specific and efficient in their ability to delivery much higher amounts of payload to target cells such as cancer.

IgG-based methods are limited by their valency, which greatly limits their ability to activate the internalization machinery on a target cell. Higher valency approaches such as IgM antibodies or engineered hexabodies are limited by short PK, large size, limited ability to control ordered and reproducible clustering and geometry of a

target or a multitude of targets. Biparatopic formats allow for binding of different domains on a target, leading to crosslinking and large clusters of bound target on the surface of a target cell. However, this tends to occur best under optimal conditions, including high target expression, which is already a limiting factor for targets beyond Her2. Such formats are also limited by poor control and reproducibility of clustering in different patients, leading to poor therapeutic index for treatment, and limited ability for multispecific targeting. Darpins, nanobodies, and bicycles are small and inexpensive to produce, but they have a short half-life, low valency, lack controlled geometry for clustering, are limited in their multispecific capabilities, and have poor target clustering. In sum, the current approaches for targeted delivery of payloads struggle to deliver enough payload into target cells to generate optimal biologic and clinical benefits.

There remains a need in the art for novel approaches that enable a dense cluster of tightly bound cell surface molecules of both the same type (multivalency) and a multitude of different targets (multispecificity) to maximize cell internalization and delivery of targeted therapeutics.

SUMMARY

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising two or more antigen-binding polypeptides and a conjugated therapeutic payload, wherein each of the two or more antigen-binding polypeptides comprises three or more antigen-binding domains, wherein each of the three or more antigen-binding domains of each of the two or more antigen-binding polypeptides can bind its respective target on a target cell, wherein the construct may bind to four or more targets on the surface of the target cell contemporaneously.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising two or more antigen-binding polypeptides and a conjugated therapeutic payload, wherein each of the two or more antigen-binding polypeptides comprises three or more antigen-binding domains, wherein each of the three or more antigen-binding domains of each of the two or more antigen-binding polypeptides can bind its respective target on a target cell, wherein the construct can bind to four or more targets on the surface of the target cell contemporaneously.

In aspects and embodiments, the construct comprises: (a) a therapeutic payload; and (b) two or more antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen binding domain (ABD A), optionally a third linker (L3), and a scaffold

region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and wherein the scaffold region comprises a ring, a central protein anchor, or an IgG Fc, or a portion of or a modified form thereof.

In aspects and embodiments, the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, wherein the construct comprises two of the antigen-binding polypeptides, wherein each of the two antigen-binding polypeptides further comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C, or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C, wherein the N- and C-terminal ABD C domains are the same or different, wherein the N- and C-terminal ABD B domains are the same or different, and wherein the N- and C-terminal ABD A domains are the same or different. Similarly, the N- and C-terminal L1 linkers can also be the same or different, wherein the N- and C-terminal L2 linkers can be the same or different, and wherein the N- and C-terminal L3 linkers can be the same or different and the N- and C-terminal hinges can also be the same or different.

In aspects and embodiments, the scaffold region comprises a rigid polypeptide; wherein the construct comprises 'n' of the antigen-binding polypeptides; wherein each of the antigen-binding polypeptides further comprises a fourth linker (L4), wherein L4 attaches the scaffold region to ABD C; and wherein the scaffold region further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring that forms a closed ring, wherein n is 3-12.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising: (a) a therapeutic payload; and (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, optionally a second L3, a second ABD A, a second L2, a second ABD B, a second L1, and a second ABD C; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 –

ABD B – L1 – ABD C or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C; and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, wherein the N- and C-terminal ABD C domains are the same or different, wherein the N- and C-terminal ABD B domains are the same or different, and wherein the N- and C-terminal ABD A domains are the same or different.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising: (a) a therapeutic payload; and (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising: (a) a therapeutic payload; and (b) four antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising: (a) a therapeutic payload; and (b) ‘n’ antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, and a fourth linker (L4); wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 optionally attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C –

L1 – ABD B – L2 – ABD A – scaffold region – L4 or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4; wherein the scaffold region comprises a rigid polypeptide; wherein the construct further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring; and wherein n is 3-12.

In aspects and embodiments, ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets a specific cell type, optionally wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell, optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell, and/or wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

In aspects and embodiments, the scaffold region comprises IgG C_H2-C_H3, or a fragment thereof, optionally wherein the IgG C_H2-C_H3 further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-C_H2-C_H3-hinge.

In aspects and embodiments, ABD C, ABD B, ABD A, L1, L2, L3, L4, hinge region, second hinge region, and/or the scaffold region comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue or an engineered glycan or tag for site specific conjugation.

In aspects and embodiments, ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule, optionally wherein the N-terminal ABD C binds to a first target molecule, the N-terminal ABD B binds to a second target molecule, the N-terminal ABD A binds to a third target molecule, the C-terminal ABD C binds to a fourth target molecule, the C-terminal ABD B binds to a fifth target molecule, and/or the C-terminal ABD A binds to a sixth target molecule. In aspects and

embodiments, ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

In aspects and embodiments, L1, L2, L3, and/or L4 is about 15-30 aa long. In aspects and embodiments, L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues. In aspects and embodiments, L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues. In aspects and embodiments, L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG C_H2-C_H3, Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15), or Gly-Gly-Ser-Asn-Gly-Ser-Gly-Gly-Ser (SEQ ID NO: 28).

In aspects and embodiments, the scaffold region comprises a rigid protein. In aspects and embodiments, the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG C_H2-C_H3, human serum albumin, human transferrin, or a fragment thereof.

In aspects and embodiments, the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

In aspects and embodiments, provided herein is a nucleic acid encoding a multivalent multispecific construct described herein.

In aspects and embodiments, provided herein is a host cell comprising a nucleic acid described herein.

In aspects and embodiments, provided herein is a pharmaceutical composition comprising a multivalent multispecific construct described herein, a nucleic acid described herein, or a host cell described herein.

In aspects and embodiments, provided herein is a method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering a multivalent

multispecific construct described herein, a nucleic acid described herein, a host cell described herein, or a pharmaceutical composition described herein.

In aspects and embodiments, the cancer is a solid tumor, optionally wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

In aspects and embodiments, the cancer is a hematological cancer, optionally wherein the cancer is leukemia, lymphoma, or multiple myeloma.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a non-limiting example of a multivalent multispecific construct described herein having two antigen-binding peptides that target two or more different targets, each comprising two copies of antigen-binding domains (ABDs) A, B, and C, two copies of linkers L1, L2, and optionally L3, and a scaffold region, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C. It should be noted that the characteristics (e.g., "rigid," "planar," etc.) are for illustration only.

Figure 2 shows a non-limiting example of a multivalent multispecific construct described herein having two antigen-binding peptides that target two or more different targets, each comprising antigen-binding domains (ABDs) A, B, and C, linkers L1, L2, and optionally L3, and a scaffold region, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region. It should be noted that the characteristics (e.g., "rigid," "planar," etc.) are for illustration only.

Figure 3 shows a non-limiting example of a multivalent multispecific construct described herein having four antigen-binding peptides that target two or more different targets, each comprising antigen-binding domains (ABDs) A, B, and C, linkers L1, L2, and optionally L3, and a scaffold region, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region. It should be noted that the size and other characteristics (e.g., "rigid," "planar," etc.) are for illustration only.

Figure 4 shows a non-limiting example of a multivalent multispecific construct described herein having 'n' antigen-binding peptides that target two or more different targets, each comprising antigen-binding domains (ABDs) A, B, and C, linkers L1, L2, optionally L3, and L4, and a scaffold region, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4 or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4, and a clasp domain that attaches the first and nth antigen-binding polypeptides to form a

closed ring. It should be noted that the binding affinities and other characteristics (e.g., "rigid," "angular," "flexible," "strong binding," "monospecific," etc.) are for illustration only. It should also be noted that the functional language (e.g., "increases half life of molecule by rescuing proteins from intracellular degradation," "allows for multispecificity," etc.) is for illustration only and without wishing to be bound by theory.

Figures 5A and 5B show binding and functional activity of multivalent constructs described herein comprising ABDs targeting OX40.

Figure 6A shows expression assembly and quality of Y6 and X12 constructs comprising VHH binding subunits targeting OX40, PD-L1 and 41BB.

Figure 6B shows activity in OX40 activation reporter assay, of multivalent multispecific constructs described herein comprising ABDs targeting OX40, PD-L1, and 4-1BB.

Figure 7 shows activity of multivalent multispecific constructs described herein comprising ABDs targeting OX40, PD-L1, and 4-1BB, and mixed multispecific constructs described herein comprising 41BB fused peptide trimers and ABDs targeting CD38 (Y12 format).

Figure 8A depicts bioconjugation of multivalent constructs with second C-terminal hinge to achieve DARs comparable to IgG – shown here in schematic with VHH-Fc as an example.

Figure 8B shows activity of multivalent constructs described herein comprising ABDs targeting OX40 and having different linkers in the inter VHH regions.

Figure 8C shows expression of constructs described herein comprising different linker-hinge combinations.

Figure 9 shows the results of an internalization assay of multivalent constructs described herein comprising ABDs targeting BCMA.

Figure 10 shows the results of an internalization assay of multivalent constructs described herein comprising ABDs targeting GPRC5D.

Figure 11 shows the results of an internalization assay of multivalent constructs described herein comprising ABDs targeting CD38.

Figure 12 shows the results of an internalization assay of multivalent multispecific constructs described herein comprising ABDs targeting BCMA, CD38, and GPRC5D.

Figure 13 shows the results of a cell killing assay including a multivalent multispecific construct described herein comprising ABDs targeting CD38, GPRC5D, and/or BCMA.

Figure 14 shows the results of an activity assay of multivalent multispecific constructs described herein comprising ABDs targeting GLP-1 and/or GCG receptors.

Figure 15 shows the internalization rates of various X12 and Y6 constructs on a cell line expressing OX40.

DETAILED DESCRIPTION

The present disclosure is based, in part, on the discovery that a versatile and multivalent platform technology can be used to integrate multiple targets and signals, enabling precise delivery of highly effective therapeutic payloads with enhanced functional capabilities. Without wishing to be bound by theory, it is thought that the multiple contemporaneous contacts of the multivalent multispecific constructs described herein with multiple targets on the surface of a target cell increases their functional effect and/or internalization efficiency.

Multivalent Multispecific Constructs

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising two or more antigen-binding polypeptides and a conjugated therapeutic payload, wherein each of the two or more antigen-binding polypeptides comprises three or more antigen-binding domains, wherein each of the three or more antigen-binding domains of each of the two or more antigen-binding polypeptides can bind its respective target on a target cell, wherein the construct can bind to four or more targets on the surface of the target cell contemporaneously.

In embodiments, the present constructs are multivalent and have 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, 10, 11, or 12 binding domains. In embodiments, the present constructs are multispecific and target 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, 10, 11, or 12 targets. In embodiments, the present constructs are multivalent and have 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, 10, 11, or 12 binding domains and the present constructs are multispecific and target 1, or 2, or 3, or 4, or 5, or 6, or 7, or 8, or 9, 10, 11, or 12 targets.

In embodiments, the present constructs have 2 binding domains and target 2 targets. In embodiments, the present constructs have 3 binding domains and target 2 targets. In embodiments, the present constructs have 4 binding domains and target 2 targets. In embodiments, the present constructs have 5 binding domains and target 2 targets. In embodiments, the present constructs have 6 binding domains and target 2 targets. In embodiments, the present constructs have 7 binding domains and target 2 targets. In embodiments, the present constructs have 8 binding domains and target 2 targets. In embodiments, the present constructs have 9 binding domains and target 2 targets. In embodiments, the present constructs have 10 binding

domains and target 2 targets. In embodiments, the present constructs have 11 binding domains and target 2 targets. In embodiments, the present constructs have 12 binding domains and target 2 targets.

In embodiments, the present constructs have 2 binding domains and target 3 targets. In embodiments, the present constructs have 2 binding domains and target 4 targets. In embodiments, the present constructs have 2 binding domains and target 5 targets. In embodiments, the present constructs have 2 binding domains and target 6 targets. In embodiments, the present constructs have 2 binding domains and target 7 targets. In embodiments, the present constructs have 2 binding domains and target 8 targets. In embodiments, the present constructs have 2 binding domains and target 9 targets. In embodiments, the present constructs have 2 binding domains and target 10 targets. In embodiments, the present constructs have 2 binding domains and target 11 targets. In embodiments, the present constructs have 2 binding domains and target 12 targets.

In embodiments, the present constructs have 2 binding domains and target 3 targets. In embodiments, the present constructs have 3 binding domains and target 3 targets. In embodiments, the present constructs have 4 binding domains and target 3 targets. In embodiments, the present constructs have 5 binding domains and target 3 targets. In embodiments, the present constructs have 6 binding domains and target 3 targets. In embodiments, the present constructs have 7 binding domains and target 3 targets. In embodiments, the present constructs have 8 binding domains and target 3 targets. In embodiments, the present constructs have 9 binding domains and target 3 targets. In embodiments, the present constructs have 10 binding domains and target 3 targets. In embodiments, the present constructs have 11 binding domains and target 3 targets. In embodiments, the present constructs have 12 binding domains and target 3 targets.

In embodiments, the present constructs have 3 binding domains and target 3 targets. In embodiments, the present constructs have 3 binding domains and target 4 targets. In embodiments, the present constructs have 3 binding domains and target 5 targets. In embodiments, the present constructs have 3 binding domains and target 6 targets. In embodiments, the present constructs have 3 binding domains and target 7 targets. In embodiments, the present constructs have 3 binding domains and target 8 targets. In embodiments, the present constructs have 3 binding domains and target 9 targets. In embodiments, the present constructs have 3 binding domains and target 10 targets. In embodiments, the present constructs have 3 binding domains and target 11 targets. In embodiments, the present constructs have 3 binding domains and target 12 targets.

In embodiments, the present constructs have 2 binding domains and target 4 targets. In embodiments, the present constructs have 3 binding domains and target 4 targets. In embodiments, the present constructs have 4 binding domains and target 4 targets. In embodiments, the present constructs have 5 binding domains and target 4 targets. In embodiments, the present constructs have 6 binding domains and target 4 targets. In embodiments, the present constructs have 7 binding domains and target 4 targets. In embodiments, the present constructs have 8 binding domains and target 4 targets. In embodiments, the present constructs have 9 binding domains and target 4 targets. In embodiments, the present constructs have 10 binding domains and target 4 targets. In embodiments, the present constructs have 11 binding domains and target 4 targets. In embodiments, the present constructs have 12 binding domains and target 4 targets.

In embodiments, the present constructs have 4 binding domains and target 3 targets. In embodiments, the present constructs have 4 binding domains and target 4 targets. In embodiments, the present constructs have 4 binding domains and target 5 targets. In embodiments, the present constructs have 4 binding domains and target 6 targets. In embodiments, the present constructs have 4 binding domains and target 7 targets. In embodiments, the present constructs have 4 binding domains and target 8 targets. In embodiments, the present constructs have 4 binding domains and target 9 targets. In embodiments, the present constructs have 4 binding domains and target 10 targets. In embodiments, the present constructs have 4 binding domains and target 11 targets. In embodiments, the present constructs have 4 binding domains and target 12 targets.

In aspects and embodiments, the construct binds to the four or more targets on the surface of the target cell within a 20-30 nm radius. Without wishing to be bound by theory, it is thought that this binding allows multiple target internalization mechanisms to increase internalization, and that this binding thus maximizes internalization of the payload. In aspects and embodiments, the multivalent multispecific construct comprises a scaffold region that comprises a rigid protein. In aspects and embodiments, the multivalent multispecific construct comprises a scaffold region that comprises IgG C_H2-C_H3, or a fragment thereof, for FcRn binding and extended PK. In aspects and embodiments, the multivalent multispecific construct comprises an FcRn binding domain or moiety, for FcRn binding and extended PK. In aspects and embodiments, the multivalent multispecific construct comprises linkers between the each of the three or more antigen-binding domains that have a rigidity to flexibility gradient going from central to distal, with the most central linker being rigid and the most distal linker being highly flexible. Without wishing to be bound by theory, it is thought that this linker configuration allows the construct to maintain a central planar interacting surface to maximize density of clustered targets, while allowing the edges can adapt to target geometry.

In aspects and embodiments, the multivalent multispecific construct comprises (a) a therapeutic payload; and (b) two or more antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or

(ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region;

and wherein the scaffold region comprises a ring, a central protein anchor, or an IgG Fc, or a portion of or a modified form thereof.

This arrangement may be referred to herein as "Y6." In aspects and embodiments, ABD C and L1 are absent. This arrangement may be referred to herein as "Y4." In aspects and embodiments, ABD C, L1, ABD B, and L2 are absent. This arrangement may be referred to herein as "Y2."

In aspects and embodiments, the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof. In aspects and embodiments, the scaffold region comprises IgG C_H2-C_H3. In aspects and embodiments, the scaffold region comprises hinge-C_H2-C_H3. In aspects and embodiments, the scaffold region comprises hinge-C_H2-C_H3-hinge. In aspects and embodiments, the scaffold region comprises a chemical core bound using click chemistry.

In aspects and embodiments, the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, and the multivalent multispecific construct comprises two of the antigen-binding polypeptides. In aspects and embodiments, each of the two antigen-binding polypeptides further comprises, arranged from N- to C-terminus,

(i) ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or

(ii) L3 – ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C.

This arrangement may be referred to herein as "X12." In aspects and embodiments, the N- and C-terminal ABD C domains are the same. In aspects and embodiments, the N- and C-terminal ABD C domains are different. In aspects and embodiments, the N- and C-terminal ABD C domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD C domains bind different targets. In aspects and embodiments, the N- and C-terminal ABD B domains are the same. In aspects and embodiments, the N- and C-terminal ABD B domains are different. In aspects and embodiments, the N- and C-terminal ABD B domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD B domains bind different targets. In aspects and embodiments, the N- and C-terminal ABD A domains are the same. In aspects and embodiments, the N- and C-terminal ABD A domains are different. In aspects and embodiments, the N- and C-terminal ABD A domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD A domains bind different targets. In aspects and embodiments, the N-terminal ABD C binds to a first target molecule, the N-terminal ABD B binds to a second target molecule, the N-terminal ABD A binds to a third target molecule, the C-terminal ABD C binds to a fourth target molecule, the C-terminal ABD B binds to a fifth target molecule, and/or the C-terminal ABD A binds to a sixth target molecule.

In aspects and embodiments, ABD C and L1 are absent. This arrangement may be referred to herein as "X8." In aspects and embodiments, ABD C, L1, ABD B, and L2 are absent. This arrangement may be referred to herein as "X4."

In aspects and embodiments, one or more of ABD A, L2, ABD B, L1 and ABD C are absent. For example, in aspects and embodiments, each of the two antigen-binding polypeptides comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B;

(ii) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A;

(iii) ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C;

(iv) ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C;

(v) ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B;

(vi) ABD B – L2 – ABD A – scaffold region – ABD A;

(vii) ABD A – scaffold region – ABD A – L2 – ABD B;

(viii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B;

(ix) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A;

(x) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C;

(xi) ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C;

(xii) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B;

(xiii) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A; or

(xiv) ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B.

These arrangements may be referred to herein as "asymmetric."

In aspects and embodiments, the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain, and the construct comprises four of the antigen-binding polypeptides.

In aspects and embodiments, the scaffold region comprises a rigid polypeptide; wherein the construct comprises 'n' of the antigen-binding polypeptides; wherein each of the antigen-binding polypeptides further comprises a fourth linker (L4), wherein L4 attaches the scaffold region to ABD C; and wherein the scaffold region further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring that forms a closed ring, wherein n is 3-12. In aspects and embodiments, the scaffold region comprises an angled pi-alpha helix framework. In aspects and embodiments, the clasp domain comprises an IgM tail.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising (a) a therapeutic payload; and (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, optionally a second L3, a second ABD A, a second L2, a second ABD B, a second L1, and a second ABD C; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or

(ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C;

and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

In aspects and embodiments, the N- and C-terminal ABD C domains are the same. In aspects and embodiments, the N- and C-terminal ABD C domains are different. In aspects and embodiments, the N- and C-terminal ABD C domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD C domains bind different targets. In aspects and embodiments, the N- and C-terminal ABD B domains are the same. In aspects and embodiments, the N- and C-terminal ABD B domains are different. In aspects and embodiments, the N- and C-terminal ABD B domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD B domains bind different targets. In aspects and embodiments, the N- and C-terminal ABD A domains are the same. In aspects and embodiments, the N- and C-terminal ABD A domains are different. In aspects and embodiments, the N- and C-terminal ABD A domains bind the same target. In aspects and embodiments, the N- and C-terminal ABD A domains bind different targets. In aspects and embodiments, the N-terminal ABD C binds to a first target molecule, the N-terminal ABD B binds to a second target molecule, the N-terminal ABD A binds to a third target molecule, the C-terminal ABD C binds to a fourth target molecule, the C-terminal ABD B binds to a fifth target molecule, and/or the C-terminal ABD A binds to a sixth target molecule.

In aspects and embodiments, one or more of ABD A, L2, ABD B, L1 and ABD C are absent. For example, in aspects and embodiments, each of the two antigen-binding polypeptides comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B;

(ii) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A;

(iii) ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C;

(iv) ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C;

(v) ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B;

(vi) ABD B – L2 – ABD A – scaffold region – ABD A;

(vii) ABD A – scaffold region – ABD A – L2 – ABD B;

(viii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B;

(ix) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A;

(x) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C;

(xi) ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C;

(xii) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B;

(xiii) ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A; or

(xiv) ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B.

In aspects and embodiments, provided herein is a multivalent multispecific construct comprising (a) a therapeutic payload; and (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or

(ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region;

and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

In aspects and embodiments, provided herein is a multispecific construct comprising (a) a therapeutic payload; and (b) four antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or

(ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region;

and wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain.

In aspects and embodiments, provided herein is a multispecific construct comprising (a) a therapeutic payload; and (b) ‘n’ antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding

domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, and a fourth linker (L4); wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 optionally attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus,

(i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4 or

(ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4;

wherein the scaffold region comprises a rigid polypeptide; wherein the construct further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring; and wherein n is 3-12.

Antigen-Binding Domains

In aspects and embodiments, the multivalent multispecific constructs provided herein comprise two or more antigen-binding polypeptides that each comprise three or more antigen-binding domains. As used herein, the term "antigen binding domain" refers to a peptide, a peptide ligand, a modified peptide, a non-natural peptide, a polypeptide, or a set of associated polypeptides that is capable of specifically binding a target molecule. In some embodiments, the "antigen binding domain" is the minimal peptide ligand sequence of a natural or synthetic peptide ligand that binds with specificity to the antigen bound by the ligand. In some embodiments, the "antigen binding domain" is the minimal sequence of an antibody that binds with specificity to the antigen bound by the antibody. In aspects and embodiments, the multivalent multispecific constructs provided herein comprise two or more antigen-binding polypeptides that each comprise one or more of a first antigen-binding domain (ABD C), a second antigen-binding domain (ABD B), and a third antigen-binding domain (ABD A). In aspects and embodiments, the multivalent multispecific constructs provided herein may bind two or more targets contemporaneously. In aspects and embodiments, binding contemporaneously means that there are no geometric or other constraints that preclude two or more ABDs present in the multivalent multispecific constructs from functionally interacting with their respective targets at the same time.

In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises any antigen-binding domain or format known in the art. Non-limiting examples of antigen-binding domains include a ligand, an extracellular domain, and engineered protein binding motif, a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, or a binding fragment of any thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a VHH or a nanobody, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD

B, and/or ABD A comprises a VH domain, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a VNAR, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises CDR1-HV2-HV4-CDR3 domains, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a Fab, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises VH-CH1-CL-VL domains, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a ScFv, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises VH-VL domains, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a diabody, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises 2x VH-VL domains, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a synthetic minibody, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises 2x VH-VL-CH3 domains, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises an extracellular domain, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a ligand, or a binding fragment thereof. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a BCMA ligand.

In aspects and embodiments, ABD C binds OX40, ABD B binds PDL1, and ABD A binds 41BB. In aspects and embodiments, ABD C binds OX40, ABD B binds 41BB, and ABD A binds PDL1.

In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a HER2 ligand. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a HER2 ligand comprising the sequence KCCYSL (SEQ ID NO: 27). In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a 41BB ligand. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a trimeric 41BB ligand. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises BAFF. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises APRIL. In aspects and embodiments, ABD C, ABD B, and/or ABD A is an immunoglobulin-based agent. In aspects and embodiments, ABD C, ABD B, and/or ABD A is an non-immunoglobulin-based agent.

In aspects and embodiments, ABD C, ABD B, and/or ABD A is a conditional binder. As used herein, the term “conditional binder” is a binder that preferentially binds under certain biological conditions, including, but not limited to, pH levels, ATP levels, calcium levels, efficient binding only when 1 or 2 or 3 or 4 other binders are bound, and protease levels.

In aspects and embodiments, binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets under acidic pH conditions. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at a pH of about 6.5 or below, about 6.4 or below, about 6.3 or below, about 6.2 or below, about 6.1 or below, about 6.0 or below, about 5.9 or below, about 5.8 or below, about 5.7 or below, about 5.6 or below, or about 5.5 or below. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets under neutral pH conditions. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at a pH of about 6.5 to about 7.5, about 6.6 to about 7.5, about 6.7 to about 7.5, about 6.8 to about 7.5, about 6.9 to about 7.5, about 7.0 to about 7.5, about 7.1 to about 7.5, about 7.2 to about 7.5, about 7.3 to about 7.5, or about 7.4 to about 7.5. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets under basic pH conditions. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at a pH of about 7.8 or above, 7.9 or above, 8.0 or above, 8.1 or above, 8.2 or above, 8.3 or above, 8.4 or above, or 8.5 or above.

In aspects and embodiments, binding of ABD C, ABD B, and/or ABD A to their respective targets depends on calcium ion concentration. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at low calcium ion concentrations. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at calcium ion concentrations of about 9 μM $CaCl_2$ or less, about 8 μM $CaCl_2$ or less, about 7 μM $CaCl_2$ or less, about 6 μM $CaCl_2$ or less, about 5 μM $CaCl_2$ or less, about 4 μM $CaCl_2$ or less, about 3 μM $CaCl_2$ or less, about 2 μM $CaCl_2$ or less, or about 1 μM $CaCl_2$ or less. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at high calcium ion concentrations. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at calcium ion concentrations of about 1 mM $CaCl_2$ or more, about 1.2 mM $CaCl_2$ or more, about 1.4 mM $CaCl_2$ or more, about 1.6 mM $CaCl_2$ or more, about 1.8 mM $CaCl_2$ or more, about 2 mM $CaCl_2$ or more, about 2.2 mM $CaCl_2$ or more, about 2.4 mM $CaCl_2$ or more, about 2.6 mM $CaCl_2$ or more, about 2.8 mM $CaCl_2$ or more, about 3 mM $CaCl_2$ or more, about 3.5 mM $CaCl_2$ or more, about 4 mM $CaCl_2$ or more, about 4.5 mM $CaCl_2$ or more, or about 5 mM $CaCl_2$ or more.

In aspects and embodiments, binding of ABD C, ABD B, and/or ABD A to their respective targets depends on protease levels and/or activity. In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises a masking moiety. In aspects and embodiments, a masking moiety is a peptide capable of binding to, or

otherwise exhibiting an affinity for, an ABD or functional fragment thereof such that the masking moiety blocks, occludes, inhibits (e.g., decreases) or otherwise prevents (e.g., masks) the ABD from engaging with its target. In aspects and embodiments, a proteolytically cleavable linker links the masking moiety to ABD C, ABD B, and/or ABD A. In aspects and embodiments, cleavage of the proteolytically cleavable linker by a protease unmasks the ABD, allowing for engagement with its target.

In aspects and embodiments, binding of ABD C, ABD B, and/or ABD A to their respective targets depends on extracellular ATP levels. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at low extracellular ATP levels. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at extracellular ATP levels of about 50 nM ATP or less, about 40 nM ATP or less, about 30 nM ATP or less, about 20 nM ATP or less, about 15 nM ATP or less, or about 10 nM ATP or less. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at high extracellular ATP levels. In aspects and embodiments, ABD C, ABD B, and/or ABD A preferentially engage with their respective targets at extracellular ATP levels of about 10 μM ATP or more, about 20 μM ATP or more, about 30 μM ATP or more, about 40 μM ATP or more, about 50 μM ATP or more, about 60 μM ATP or more, about 70 μM ATP or more, about 80 μM ATP or more, about 90 μM ATP or more, or about 100 μM ATP or more.

In aspects and embodiments, the multivalent multispecific constructs provided herein can be used to target their therapeutic payload to any desired eukaryotic cell, referred to herein as a "target cell." As used herein, a "target cell" refers to any cell expressing one or more antigens recognized by ABD C, ABD B, and/or ABD A. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets a specific cell type. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets a mammalian cell (e.g., a cell of a rodent, a mouse, a rat, an ungulate, a cow, a sheep, a pig, a horse, a camel, a rabbit, a canine (dog), a feline (cat), a primate, a non-human primate, or a human). In aspects and embodiments, ABD C, ABD B, and/or ABD A targets bone marrow cells, hematopoietic stem cells (HSCs), long-term HSCs, short-term HSCs, hematopoietic stem and progenitor cells (HSPCs), peripheral blood mononuclear cells (PBMCs), myeloid progenitor cells, lymphoid progenitor cells, T-cells, B-cells, NKT cells, NK cells, dendritic cells, monocytes, granulocytes, erythrocytes, megakaryocytes, mast cells, basophils, eosinophils, neutrophils, macrophages, erythroid progenitor cells (e.g., HUDEP cells), megakaryocyte-erythroid progenitor cells (MEPs), common myeloid progenitor cells (CMPs), multipotent progenitor cells (MPPs), long term HSCs (LT-HSCs), endothelial cells, neurons, astrocytes, pancreatic cells, pancreatic β-islet cells, muscle cells, skeletal muscle cells, cardiac muscle cells, hepatic cells, fat cells, intestinal cells, cells of the colon, cells of the stomach. In aspects and embodiments,

ABD C, ABD B, and/or ABD A targets diseased cells. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets cancer cells. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets solid cancer cells. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets hematological cancer cells. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets pathogen-infected cells, including, but not limited to, cells infected by a bacteria, a fungus, or a virus. In aspects and embodiments, ABD C, ABD B, and/or ABD A targets cancer cells, neural cells, stem cells, cells for regenerative medicine, or immune cells.

In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell by promoting clathrin-mediated endocytosis of the multivalent multispecific construct by the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell by promoting receptor-mediated endocytosis of the multivalent multispecific construct by the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell by binding to one or more antigens selected from IGF2R, CASP9 domains, endosomal degradative subdomains, and retrieval subdomains.

In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell by binding to one or more antigens selected from apoptosis targets, chemotherapy efflux pumps, PGP, MRP, BCRP/ABCG2, MDR, and microRNA.

In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell by binding to one or more antigens selected from DR5, DR4, FAS, TNFR1, and IAP antagonists.

In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes stimulation of immune cells, e.g., by binding to one or more of PD1, PDL1, CTLA4, LAG3, 41BB, and OX40, etc. In aspects and embodiments, ABD C, ABD B, and/or ABD A promotes rapid internalization and lysosomal localization into the target cell, e.g., by binding to one or more of CD63, PRLR, and APLP2. In aspects and embodiments, ABD C, ABD B, and/or ABD A binds and sequesters soluble factors, e.g., VEGF, TGFbeta, etc.

In aspects and embodiments, ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule. "Half-life extending molecule" as used herein refers to a molecule that when attached (e.g., covalently) to a second molecule, extends the half-life of the second molecule. Non-limiting examples of half-life extending molecules include an Fc domain, a modified Fc domain carrying mutations such as YTE, mutations that reduce FcγR binding, and/or mutations that reduce FcγRIII binding, etc., to further extend in vivo half-life, a human serum albumin (HSA), an HSA binding molecule, polyethylene glycol (PEG), polypropylene glycol (PPG), an FcRn binding domain, and XTEN. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises an FcRn binding domain. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises albumin. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises a human serum albumin (HSA). In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises PEG. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises XTEN. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises an HSA binding domain. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises PPG. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises an Fc domain. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises a modified Fc domain carrying a YTE mutation. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises a modified Fc domain carrying a mutation that reduces FcγR binding. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold comprises a modified Fc domain carrying a mutation that reduce FcγRIII binding.

In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold and/or linkers comprises at least one site for conjugation of the payload. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold and/or linkers comprises one or more Cys residues that can be used for conjugation of the payload. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold and/or linkers comprises one or more Lys residues that can be used for conjugation of the payload. In aspects and embodiments, ABD C, ABD B, and/or ABD A and/or scaffold and/or linkers comprises one or more Tyr residues that can be used for conjugation of the payload. In aspects and embodiments, L1, L2, L3, and/or L4 comprises one or more residues or glycans for site specific conjugation with payloads.

In aspects and embodiments, ABD C binds to a first target molecule, ABD B binds to a second target molecule, and ABD A binds to a third target molecule. In aspects and embodiments, ABD C and ABD B bind to a first target molecule, and ABD A binds to a second target molecule. In aspects and embodiments, ABD C and ABD A bind to a first target molecule, and ABD B binds to a second target molecule. In aspects and

embodiments, ABD B and ABD A bind to a first target molecule, and ABD C binds to a second target molecule. In aspects and embodiments, ABD C, ABD B, and ABD A bind to a first target molecule.

In aspects and embodiments, ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

In aspects and embodiments, ABD A serves as a primary anchor for binding to a target cell. As used herein, a "primary anchor" refers to the target molecule that is most highly and/or frequently expressed on the target cell of the two or more target molecules of the multivalent multispecific construct. In aspects and embodiments, ABD A binds to a target molecule that is more highly and/or more frequently expressed on the target cell than the target molecule that ABD B and/or ABD C binds. Without wishing to be bound by theory, it is thought that ABD A serving as a primary anchor for binding to a target cell prioritizes the strongest potential target antigen, i.e., the target molecule that is more highly and/or more frequently expressed on the target cell. In aspects and embodiments, ABD A serving as a primary anchor for binding to a target cell allows for conditional binding of ABD B and/or ABD C to their respective targets when ABD A is bound to its target. In aspects and embodiments, ABD C serves as a secondary anchor for binding to a target cell. As used herein, a "secondary anchor" refers to the target molecule that is second-most highly and/or frequently expressed on the target cell of the two or more target molecules of the multivalent multispecific construct. In aspects and embodiments, ABD C binds to a target molecule that is less highly and/or more frequently expressed on the target cell than the target molecule that ABD A binds but more highly and/or more frequently expressed on the target cell than the target molecule that ABD B binds. In aspects and embodiments, ABD C serving as a secondary anchor for binding to a target cell allows for conditional binding of ABD B to its target when ABD C is bound to its target. In aspects and embodiments, ABD B only substantially stably engages with its target when ABD A is bound to its target and/or ABD C is bound to its target.

In aspects and embodiments, ABD C serves as a primary anchor for binding to a target cell. As used herein, a "primary anchor" refers to the target molecule that is most highly and/or frequently expressed on the target cell of the two or more target molecules of the multivalent multispecific construct. In aspects and embodiments, ABD C binds to a target molecule that is more highly and/or more frequently expressed on the target cell than the target molecule that ABD B and/or ABD A binds. Without wishing to be bound by theory, it is thought that ABD C serving as a primary anchor for binding to a target cell, in some cases, prioritizes the strongest potential target antigen, i.e., the target molecule that is more highly and/or more frequently expressed on the target cell. In aspects and embodiments, ABD C serving as a primary anchor for binding to

a target cell allows for conditional binding of ABD B and/or ABD A to their respective targets when ABD C is bound to its target. In aspects and embodiments, ABD A or ABD B serves as a secondary anchor for binding to a target cell. As used herein, a "secondary anchor" refers to the target molecule that is second-most highly and/or frequently expressed on the target cell of the two or more target molecules of the multivalent multispecific construct. In aspects and embodiments, ABD A and/or B binds to a target molecule that is less highly and/or more frequently expressed on the target cell than the target molecule that ABD C binds but more highly and/or more frequently expressed on the target cell than the target molecule that ABD B or A binds. In aspects and embodiments, ABD A or B serving as a secondary anchor for binding to a target cell allows for conditional binding of remaining ABDs B (or A) to its target when ABD C is bound to its target. In aspects and embodiments, ABD B (or A) only substantially stably engages with its target when ABD A is bound to its target and/or ABD C is bound to its target.

Cellular Targets

In aspects and embodiments, the multivalent multispecific constructs provided herein can be directed to any desired cellular target. Non-limiting examples of cellular targets include NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, Her2, EGFR, PDL1, CEA, DLL3, CD47, Her3, FolRa, DR5, mesothelin, CEACAM5, CD38, CD19, ALPL2, PRLR, Integrin, CD20, FcRn, FcRH5, GPRC5D, Her2, Her3, OX40, 41BB, and CD79b.

In aspects and embodiments, ABD A binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, Her2, EGFR, PDL1, CEA, FolRa, DR5, mesothelin, CEACAM5, CD38, CD19, ALPL2, PRLR, Integrin, CD20, FcRn, FcRH5, GPRC5D, Her2, Her3, OX40, 41BB, and CD79b. In aspects and embodiments, ABD B binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, Her2, EGFR, PDL1, CEA, FolRa, DR5, mesothelin, CEACAM5, CD38, CD19, ALPL2, PRLR, Integrin, CD20, FcRn, FcRH5, GPRC5D, Her2, Her3, OX40, 41BB, and CD79b. In aspects and embodiments, ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, Her2, EGFR, PDL1, CEA, FolRa, DR5, mesothelin, CEACAM5, CD38, CD19, ALPL2, PRLR, Integrin, CD20, FcRn, FcRH5, GPRC5D, Her2, Her3, OX40, 41BB, and CD79b.

In aspects and embodiments, one of ABD C, ABD B, and ABD A binds Her2, and one of ABD C, ABD B, and ABD A binds Trop2 and/or Her3.

In aspects and embodiments, ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

In aspects and embodiments, ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds FolRa. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

In aspects and embodiments, ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of NSCLC.

In aspects and embodiments, ABD C binds B7H3, ABD B binds EGFR, and ABD A binds Trop2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of NSCLC.

In aspects and embodiments, ABD C binds B7H3, ABD B binds EGFR, and ABD A binds Trop2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of NSCLC.

In aspects and embodiments, ABD C binds DLL3, ABD B binds B7H3, and ABD A binds Trop2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of SCLC.

In aspects and embodiments, ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of GI cancer.

In aspects and embodiments, ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of myeloma.

In aspects and embodiments, ABD C binds BCMA, ABD B binds CD38, and ABD A binds GPRC5D. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of myeloma.

In aspects and embodiments, ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

In aspects and embodiments, ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of breast cancer.

In aspects and embodiments, ABD C binds GPRC5D and ABD A binds BCMA. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of myeloma.

In aspects and embodiments, ABD C binds CD22 and ABD A binds CD79b. In aspects and embodiments, the multivalent multispecific construct is suitable for treatment of a B cell malignancy.

In aspects and embodiments, ABD C binds OX40, ABD B binds PDL1, and ABD A binds 41BB. In aspects and embodiments, ABD C binds OX40, ABD B binds 41BB, and ABD A binds PDL1.

In aspects and embodiments, ABD C comprises a 41BB ligand, and ABD B, and/or ABD A comprises a HER2 ligand. In aspects and embodiments, ABD C comprises a trimeric 41BB ligand, and ABD B and ABD A each comprises a HER2 ligand. In aspects and embodiments, ABD C comprises a trimeric 41BB ligand, and ABD B and ABD A each comprises a HER2 ligand comprising the sequence KCCYSL (SEQ ID NO: 27).

Linkers

In aspects and embodiments, the multivalent multispecific constructs described herein comprise linkers between the antigen-binding domains, and between the antigen-binding domains and the scaffold regions. The term "linker," as used herein, refers to a molecule linking two other molecules or moieties. In aspects and embodiments, the multivalent multispecific constructs described herein comprise a first linker (L1), a second linker (L2), optionally a third linker (L3), and optionally a fourth linker (L4). In aspects and embodiments, L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and ABD A is attached to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region. In aspects and embodiments, L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region. In aspects and embodiments, each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C. In aspects and embodiments, each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C. In aspects and embodiments, L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, ABD A is attached to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4. In aspects and embodiments, L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4.

In aspects and embodiments, L1, L2, L3, and/or L4 is about 4-50 aa long, about 10-45 aa long, about 15-40 aa long, about 15-35 aa long, or about 15-30 aa long. In aspects and embodiments, L1, L2, L3, and/or L4 is

about 4 aa long, about 5 aa long, about 6 aa long, about 7 aa long, about 8 aa long, about 9 aa long, about 10 aa long, about 11 aa long, about 12 aa long, about 13 aa long, about 14 aa long, about 15 aa long, about 16 aa long, about 17 aa long, about 18 aa long, about 19 aa long, about 20 aa long, about 21 aa long, about 22 aa long, about 23 aa long, about 24 aa long, about 25 aa long, about 26 aa long, about 27 aa long, about 28 aa long, about 29 aa long, about 30 aa long, about 31 aa long, about 32 aa long, about 33 aa long, about 34 aa long, about 35 aa long, about 36 aa long, about 37 aa long, about 38 aa long, about 39 aa long, about 40 aa long, about 41 aa long, about 42 aa long, about 43 aa long, about 44 aa long, about 45 aa long, about 46 aa long, about 47 aa long, about 48 aa long, about 49 aa long, or about 50 aa long.

In aspects and embodiments, L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues. In aspects and embodiments, L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues. In aspects and embodiments, L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

In aspects and embodiments, L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

In aspects and embodiments, L1, L2, L3, and/or L4 is hydrophilic. Hydrophobicity values are determined according to methods known in the art. In aspects and embodiments, hydrophobicity values are calculated using the normalized hydrophobicity scale of Eisenberg, Ann. Rev. Biochem. 53:595-623, 1984. In aspects and embodiments, L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, about 0.5 to about 0.7, or about 0.6 to about 0.65. In aspects and embodiments, L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4, about 0.45, about 0.5, about 0.55, about 0.6, about 0.65, about 0.7, about 0.75, or about 0.8.

In aspects and embodiments, L1, L2, L3, and/or L4 is substantially non-immunogenic. As used herein, "non-immunogenic" refers to a characteristic of a composition which, when administered to a mammal such as a human, are not associated with the development of a humoral (antibody) and/or or cellular immune response.

In aspects and embodiments, L1, L2, L3, and/or L4 is substantially non-cleavable. As used herein, "non-cleavable linker" refers to linkers where the release of the therapeutic payload does not depend on, for

example, the differential properties between the plasma and some cytoplasmic compartments, or whether the linker has a physical property that permits enzymatic cleavage or chemical cleavage.

In aspects and embodiments, L1, L2, L3, and/or L4 is substantially cleavable. As used herein, "cleavable linker" refers to a linker that can connect two or more molecules and then be cleaved once exposed to an agent. Cleavable linkers can include chemically or enzymatically unstable or degradable linkages. In aspects and embodiments, cleavable linkers can rely on processes inside the cell to release the therapeutic payload, such as reduction in the cytoplasm, exposure to acidic conditions in the lysosome, or cleavage by specific proteases or other enzymes within the cell. In aspects and embodiments, cleavable linkers incorporate one or more chemical bonds that are either chemically or enzymatically cleavable while the remainder of the linker is non-cleavable. Such linkers can also be intrinsically sensitive to their environment or signals, which can lead to changes in their biophysical properties.

In aspects and embodiments, L1, L2, L3, and/or L4 is about 4-30 nm long, about 5-25 nm long, about 5-20 nm long, about 5-15 nm long, or about 5-10 nm long. In aspects and embodiments, L1, L2, L3, and/or L4 is about 5 nm long, about 6 nm long, about 7 nm long, about 8 nm long, about 9 nm long, about 10 nm long, about 12 nm long, about 14 nm long, about 16 nm long, about 18 nm long, about 20 nm long, about 22 nm long, about 24 nm long, about 26 nm long, about 28 nm long, or about 30 nm long.

In aspects and embodiments, L2 and/or L3 is a rigid linker. As used herein, "rigid" linker refers to a molecule that adopts a relatively well-defined conformation when in solution. In aspects and embodiments, rigid linkers are those which have a particular secondary and/or tertiary structure in solution. In aspects and embodiments, rigid linkers include aromatic molecules (see, e.g., U.S. Pat. No. 6,096,875 or U.S. Pat. No. 5,948,648), peptide linkers rich in proline, or peptide linkers having an inflexible helical structure. Rigid linkers are described in, for example, Chen, et al. (2013) Adv. Drug Deliv. Rev. 65(10):1357-1369; US 2010/0158823 and US 2009/10221477, each of which is incorporated herein by reference in its entirety. In aspects and embodiments, rigid linkers comprise helical structures. In aspects and embodiments, rigid linkers are rich in Pro. In aspects and embodiments, L3 is about 5-15 nm long, about 6-4 nm long, or about 7-12 nm long. In aspects and embodiments, L3 comprises an alpha helix.

In aspects and embodiments, L1 is a flexible linker. As used herein, "flexible linker" refers to a hydrocarbon or peptide linker that does not have a fixed structure (secondary or tertiary structure) in solution. In aspects and embodiments, flexible linkers include hydrocarbon linkers and peptide linkers composed of small, non-polar. (e.g., Gly) and/or polar (e.g., Ser or Thr) amino acid residues. In aspects and embodiments, flexible

linkers comprise simple amino acids (e.g., amino acids with simple side chains (e.g., H, CH3 or CH2OH)), as the lack of branched side chains on these amino acids provides greater flexibility (e.g., two-dimensional or three-dimensional flexibility) within the linker and, accordingly, within a polypeptide composition. In aspects and embodiments, flexible linkers contain additional amino acids such as Thr and Ala to maintain flexibility, as well as polar amino acids such as Lys and Glu to improve solubility. Flexible linkers are described, for example, in Chen, et al. (2013) Adv. Drug Deliv. Rev. 65(10):1357-1369; US 2012/0232021; US 2014/0079701; WO 1999/045132; WO 1994/012520 and WO 2001/1053480, each of which is incorporated herein by reference in its entirety. In aspects and embodiments, L1 is about 5-15 nm long, about 6-4 nm long, or about 7-12 nm long. In aspects and embodiments, L1 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

In aspects and embodiments, L2 is a moderately rigid linker. Any moderately rigid linkers known in the art can be used herein. In aspects and embodiments, L2 is about 5-15 nm long, about 6-4 nm long, or about 7-12 nm long. In aspects and embodiments, L2 comprises b2-microglobulin, Zn-a2-glycoprotein, tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity. In aspects and embodiments, L2 comprises a cleavable linker.

In aspects and embodiments, linker rigidity is determined using modeling techniques known in the art. In aspects and embodiments, linker rigidity is determined using electron microscopy techniques and Artificial Intelligence and Machine Learning modeling known in the art to show flexibility in the structure.

In aspects and embodiments, the linkers, in order from most to least rigid, are L4, L3, L2, and L1. Without wishing to be bound by theory, it is thought that this arrangement of linkers allows the multivalent multispecific construct to maintain a central planar interacting surface to maximize density of clustered targets, while allowing the more distal binders to adapt to target geometry.

Scaffold Regions

In aspects and embodiments, the scaffold region of the multivalent multispecific constructs described herein comprises a rigid protein.

In aspects and embodiments, the scaffold region of the multivalent multispecific constructs described herein comprises a moiety for extending PK. Non-limiting examples of moieties that can be used for extending PK include FcRn binding domains, IgG C_H2-C_H3 domains, human serum albumin, and human transferrin. In aspects and embodiments, the scaffold region of the multivalent multispecific constructs described herein comprises an FcRn binding domain, an IgG C_H2-C_H3, a human serum albumin, a human transferrin, or a fragment thereof.

In aspects and embodiments, the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof. In aspects and embodiments, the IgG Fc is Fc-null or -silenced. In aspects and embodiments, the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain. In aspects and embodiments, the scaffold region comprises IgG C_H2-C_H3, or a fragment thereof. In aspects and embodiments, IgG C_H2-C_H3 binds to FcRn and extends the PK of the multivalent multispecific construct. In aspects and embodiments, the scaffold region further comprises a second hinge region. In aspects and embodiments, the scaffold region further comprises hinge-C_H2-C_H3-hinge.

Hinges

In aspects and embodiments, the multivalent multispecific constructs described herein comprise a hinge region. In aspects and embodiments, the multivalent multispecific constructs described herein comprise a hinge region both at the N- and C- terminus of the scaffold. As used herein, the term "hinge region" refers to a short, flexible amino acid sequence. In aspects and embodiments, a hinge region is derived from an IgG sequence or from an interdomain region of a transmembrane protein. In aspects and embodiments, the hinge region comprises one or more of DKTHTCPPCPAPELLGG (SEQ ID NO: 16), DKTHTCPPCP (SEQ ID NO: 17), CPPC (SEQ ID NO: 18), GGLLEPAPCPPCTHTKD (SEQ ID NO: 19), PPCPSCPAPEFLGG (SEQ ID NO: 20), PPCPSCP (SEQ ID NO: 21), CPSCP (SEQ ID NO: 22), GGLFEPAPCSPCPP (SEQ ID NO: 23), GGGGDKTHTCPPCPAPELLGG (SEQ ID NO: 24), $(G_2S)_3$ (SEQ ID NO: 25), and GGGG (SEQ ID NO: 26). In aspects and embodiments, the first or second hinge comprises one or more residues or glycans for site specific conjugation with payloads. Without wishing to be bound by theory, it is thought that inclusion of a second hinge region at the C-terminus of the scaffold region allows for conjugation to inter-chain cysteine residues after limited reduction, enabling higher drug to antibody ration (DAR) than otherwise feasible in the absence of a second hinge.

Therapeutic Payloads

In aspects and embodiments, the multivalent multispecific constructs described herein comprise one or more therapeutic payloads. As used herein, the term "therapeutic payload" refers to a molecule that has a biological, a cytotoxic, or a therapeutic effect in a cell. In aspects and embodiments, the therapeutic payload can be an inorganic molecule, an organic molecule, a small molecule, a drug compound, a peptide, a polypeptide, a degrader, a biologic modulator, an immune stimulatory motif, an immune suppressant, a steroid, an antibiotic, or an antiviral. In aspects and embodiments, the therapeutic payload can be a functional nucleic acid, such as an oligonucleotide or a polynucleotide, such as an mRNA or an siRNA. In aspects and embodiments, the therapeutic payload can be an mRNA encoding a functional peptide or polypeptide, such as a gene therapy or a gene modification system. In aspects and embodiments, the multivalent multispecific constructs described herein comprise dual payloads. the multivalent multispecific constructs described herein comprise multi-payloads.

In aspects and embodiments, the therapeutic payload comprises a protein. In aspects and embodiments, the therapeutic payload comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

In aspects and embodiments, the therapeutic payload comprises a small molecule. In aspects and embodiments, the therapeutic payload comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist, or other related or combined payloads.

In aspects and embodiments, the therapeutic payload comprises an RNA molecule. In aspects and embodiments, the therapeutic payload comprises an siRNA molecule. In aspects and embodiments, the therapeutic payload comprises an mRNA molecule. In aspects and embodiments, the mRNA molecule encodes a therapeutic peptide or protein.

In aspects and embodiments, the therapeutic payload comprises a chemosensitizer. In aspects and embodiments, the therapeutic payload comprises a genetic modifier.

In aspects and embodiments, the therapeutic payload comprises a belotecan derivative attached to the scaffold region with a cathepsin-cleavable linker, a non-cleavable linker, or a partially cleavable linker.

In aspects and embodiments, the multivalent multispecific construct comprises more than one payload. In aspects and embodiments, the multivalent multispecific construct has a drug to antibody ratio (DAR) of about 1-10, about 2-9, about 2-8, about 3-8, about 4-7, or about 5-6. In embodiments, the multivalent multispecific construct has a DAR of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, or more.

Conjugation Sites and Methods

In aspects and embodiments, the multivalent multispecific constructs described herein comprise a therapeutic payload that is conjugated one or more of the antigen-binding polypeptides. Methods of conjugation are well known in the art, and any known conjugation method can be used herein.

In aspects and embodiments, the multivalent multispecific constructs described herein comprise a therapeutic payload that is conjugated one or more of the antigen-binding polypeptides through an amine linkage at one or more surface-exposed lysine residues, through a sulfide linkage at one or more surface-exposed cysteine residues, or through the primary amide side chain of Gln 295 within a deglycosylated antibody or fragment thereof. In aspects and embodiments, the therapeutic payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region. In aspects and embodiments, the therapeutic payload is conjugated to Gln 295.

In aspects and embodiments, the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

In aspects and embodiments, the payload is conjugated to the multivalent multispecific construct using stochastic conjugation by limited reduction of interchain cysteines. In aspects and embodiments, the conjugation comprises use of at least one Fc unit with intact disulfides within the hinge region. In aspects and embodiments, the conjugation comprises partial reduction with TCEP. In aspects and embodiments, the conjugation comprises conjugation of reduced cysteines with standard maleimide-based chemistry. In embodiments, the DAR of the multivalent multispecific construct is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9 or about 10.

In aspects and embodiments, the payload is conjugated to the multivalent multispecific construct using glycan conjugation of the native Fc glycan or a glycan engineered at another site. In aspects and embodiments, the payload is conjugated to the multivalent multispecific construct using glycan conjugation of one or more

engineered glycans in the inter VHH linkers. In embodiments, the DAR of the multivalent multispecific construct is about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9 or about 10.

In aspects and embodiments, the payload is conjugated to specific glutamines of to the multivalent multispecific construct using bacterial transglutaminase. In aspects and embodiments, the payload is conjugated to the multivalent multispecific construct transglutaminase conjugation to a native glutamines (Gln 295) or by insertion of a LLQG tag site.

In aspects and embodiments, the payload is conjugated to specific residues of to the multivalent multispecific construct by way of insertion of a specific CxPxR tag allow formylglycine generating enzyme (FGE) based hydrazino-Pictet-Spengler (HIPS) conjugation.

In aspects and embodiments, the payload is conjugated to specific lysines of to the multivalent multispecific construct using AjiCap technology. In aspects and embodiments, the payload is conjugated to specific glutamines of to the multivalent multispecific construct using site specific conjugation on one or more cysteines using peptide based Fc binder. In embodiments, the DAR of the multivalent multispecific construct is about 1, about 2, about 3, or about 4.

Other Components and Features

In aspects and embodiments, the multivalent multispecific construct further comprises one or more enhancer domains.

In aspects and embodiments, the multivalent multispecific construct further comprises one or more FcRn binding domain(s). In aspects and embodiments, the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

In aspects and embodiments, the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm. In aspects and embodiments, the diameter of the multivalent multispecific construct is about 5 nm, about 10 nm, about 15 nm, about 20 nm, about 25 nm, about 30 nm, about 35 nm, about 40 nm, about 45 nm, or about 50 nm.

In aspects and embodiments, each antigen-binding polypeptide of the multivalent multispecific construct contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Methods for cyclization are known in the art. See, e.g., Hayes et al., "Approaches for peptide and protein cyclisation," *Org Biomol Chem*. 2021 May 12;19(18):3983-4001, incorporated by reference herein in its entirety. Non-limiting methods for cyclization include chemical approaches and enzymatic and protein tag

approaches. Non-limiting examples of chemical approaches for cyclization include direct coupling, native chemical ligation, Syr/Thr ligation, CyClick, traceless Staudinger ligation, KAHA (type I and II), TAMM, Cu-catalysed azide–alkyne cycloaddition (CuAAC), strain promoted azide–alkyne cycloaddition (SPAAC), Cysteine disulphides, orthogonal disulphide pairing, and disulphide stapling. Non-limiting examples of enzymatic and protein tag approaches for cyclization include subtiligase variants, sortases, asparaginyl endopeptidase – butelase 1, asparaginyl endopeptidase – OaAEP1, microbial transglutaminase (*S. mobaraensis*), protein tag – intein, protein tag – SpyTag/SpyCatcher, protein tag – SpyLigase, protein tag – SnoopTag/SnoopCatcher, and protein tag – SnoopLigase.In aspects and embodiments, the multivalent multispecific constructs described herein deliver payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

In aspects and embodiments, the multivalent multispecific constructs described herein are internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A. In embodiments, the multivalent multispecific constructs described herein have improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, even when only a single target of the multivalent multispecific constructs is expressed on the target cells.

In aspects and embodiments, the multivalent multispecific constructs described herein have improved specificity for binding to target cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A. In aspects and embodiments, the multivalent multispecific constructs described herein have improved specificity for being internalized by target cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A. In aspects and embodiments, the multivalent multispecific constructs described herein have improved specificity for delivering their therapeutic payloads to target cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A. In aspects and embodiments, the multivalent multispecific constructs described herein have improved specificity for killing cancer or tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Evaluation of Constructs

In embodiments, assays are carried out to evaluate therapeutic payload delivery (e.g., specificity of binding to target cells, affinity for target cells, internalization efficacy, etc.) and therapeutic payload efficacy (e.g.,

depending on the payload, levels of cell killing, cytotoxicity, apoptosis induction, cell stimulation, gene editing, RNAi silencing, and mRNA expression, etc.) of multivalent multispecific constructs described here.

Methods of evaluating binding specificity of a multivalent multispecific construct are known. Non-limiting examples of assays used to evaluate binding affinity of multivalent multispecific constructs to their targets and target cells include ELISA assays, surface plasmon resonance assays, Western blot analysis, flow cytometry analysis, and immunohistochemistry.

Methods of evaluating internalization of a multivalent multispecific construct are known. Non-limiting examples of assays used to evaluate internalization of multivalent multispecific constructs to their targets and target cells include flow cytometry assays and microscopy methods.

Methods of determining whether a multivalent multispecific construct exerts a cytostatic and/or cytotoxic effect on a cell are known. For example, the cytotoxic or cytostatic activity of a multivalent multispecific construct can be measured by, e.g., exposing mammalian cells expressing a target antigen of the multivalent multispecific construct in a cell culture medium; culturing the cells for a period from about 6 hours to about 6 days; and measuring cell viability (e.g., using a CellTiter-Glo® (CTG) or MTT cell viability assay). In embodiments, cell-based in vitro assays may also be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the multivalent multispecific construct.

In embodiments, for determining cytotoxicity, necrosis or apoptosis (programmed cell death) may be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane, swelling of the cell, and rupture of the plasma membrane. Apoptosis can be quantitated, for example, by measuring DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica (1999) 2:34-7 (Roche Molecular Biochemicals).

In embodiments, apoptosis may also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al., eds. (1992) pp.3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Apoptosis may also be determined, in some

embodiments, by screening for caspase activity. In embodiments, a Caspase- Glo® Assay can be used to measure activity of caspase-3 and caspase-7. In embodiments, the assay provides a luminogenic caspase-3/7 substrate in a reagent optimized for caspase activity, luciferase activity, and cell lysis. In embodiments, adding Caspase-Glo® 3/7 Reagent in an "add-mix-measure" format may result in cell lysis, followed by caspase cleavage of the substrate and generation of a "glow-type" luminescent signal, produced by luciferase. In embodiments, luminescence may be proportional to the amount of caspase activity present, and can serve as an indicator of apoptosis. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage. In embodiments, determination of any of these effects on cancer cells indicates that a multivalent multispecific construct is useful in the treatment of cancers.

In embodiments, cell viability may be measured, e.g., by determining in a cell the uptake of a dye such as neutral red, trypan blue, Crystal Violet, or ALAMAR™ blue (see, e.g., Page et al. (1993) Intl J Oncology 3:473-6). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. In embodiments, cell viability may also be measured, e.g., by quantifying ATP, an indicator of metabolically active cells. In embodiments, in vitro potency and/or cell viability of prepared ADCs or Bcl-xL inhibitor compounds may be assessed using a CellTiter-Glo® (CTG) cell viability assay. In this assay, in some embodiments, the single reagent (CellTiter-Glo® Reagent) is added directly to cells cultured in serum-supplemented medium. The addition of reagent results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. In embodiments, cell viability may also be measured, e.g., by measuring the reduction of tetrazolium salts. In embodiments, in vitro potency and/or cell viability of prepared ADCs or Bcl-xL inhibitor compounds may be assessed using an MTT cell viability assay, as described in the examples provided herein. In this assay, in some embodiments, the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can then be solubilized and quantified by spectrophotometric means.

Methods of evaluating gene editing, RNAi silencing, and mRNA expression efficiency of multivalent multispecific constructs are known. Non-limiting examples of evaluating gene editing, RNAi silencing, and mRNA expression efficiency of multivalent multispecific constructs include sequencing-based analysis and qPCR analysis.

Methods of Treatment

In aspects and embodiments, provided herein is a method of treating a disease or disorder in a subject in need thereof comprising administering a multivalent multispecific construct described herein. In aspects and embodiments, the disease or disorder is a cancer. In aspects and embodiments, the disease or disorder is an immune disease. In aspects and embodiments, the disease or disorder is an autoimmune disease. In aspects and embodiments, the disease or disorder is a neurological disease. In aspects and embodiments, the disease or disorder is a cardiovascular disease. In aspects and embodiments, the disease or disorder is a dermatologic disease. In aspects and embodiments, the disease or disorder is an ocular disease. In aspects and embodiments, the disease or disorder is an infectious disease. In aspects and embodiments, the disease or disorder is one that could benefit from regenerative medicine. In aspects and embodiments, the disease or disorder is a genetic disease or disorder.

In aspects and embodiments, the disease or disorder is a cancer and the cancer is a solid tumor. In aspects and embodiments, the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

In aspects and embodiments, the disease or disorder is a cancer and the cancer is a hematological cancer. In aspects and embodiments, the cancer is leukemia, lymphoma, or multiple myeloma.

In aspects and embodiments, provided herein is a method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

In aspects and embodiments, provided herein is a method of treating NSCLC in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

In aspects and embodiments, provided herein is a method of treating GI cancer in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

In aspects and embodiments, provided herein is a method of treating myeloma in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

In aspects and embodiments, provided herein is a method of treating myeloma in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds GPRC5D and ABD A binds BCMA.

In aspects and embodiments, provided herein is a method of treating a B cell malignancy in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds CD22 and ABD A binds CD79b.

In aspects and embodiments, provided herein is a method of treating NHL or autoimmunity in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

In aspects and embodiments, provided herein is a method of treating breast cancer in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

In aspects and embodiments, provided herein is a method of treating cancer in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds OX40, ABD B binds PDL1, and ABD A binds 41BB.

In aspects and embodiments, provided herein is a method of treating cancer in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C binds OX40, ABD B binds 41BB, and ABD A binds PDL1.

In aspects and embodiments, provided herein is a method of treating cancer in a subject in need thereof comprising administering a multivalent multispecific construct described herein, wherein ABD C comprises a 41BB ligand, and ABD B, and/or ABD A comprises a HER2 ligand. In aspects and embodiments, ABD C comprises a trimeric 41BB ligand, and ABD B and ABD A each comprises a HER2 ligand. In aspects and embodiments, ABD C comprises a trimeric 41BB ligand, and ABD B and ABD A each comprises a HER2 ligand comprising the sequence KCCYSL (SEQ ID NO: 27).In aspects and embodiments, provided herein is a nucleic acid encoding a multivalent multispecific construct described herein. In aspects and embodiments, provided herein is a host cell comprising a multivalent multispecific construct described herein. In aspects and embodiments, provided herein is a host cell comprising a nucleic acid described herein. In aspects and embodiments, provided herein is a pharmaceutical composition comprising a multivalent multispecific construct, a nucleic acid, or a host cell described herein.

In aspects and embodiments, provided herein is a method of making a multivalent multispecific construct described herein comprising culturing a cell expressing the multivalent multispecific construct; and (b) isolating the multivalent multispecific construct from the cultured cell.

Definitions

In general, terms used in the claims and the specification are intended to be construed as having the plain meaning understood by a person of ordinary skill in the art. Certain terms are defined below to provide additional clarity. In case of conflict between the plain meaning and the provided definitions, the provided definitions are to be used.

Any terms not directly defined herein shall be understood to have the meanings commonly associated with them as understood within the art of the invention. Certain terms are discussed herein to provide additional guidance to the practitioner in describing the compositions, the devices, the methods and the like of aspects of the invention and how to make or use them. It will be appreciated that the same thing may be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein. No significance is to be placed upon whether or not a term is elaborated or discussed herein. Some synonyms or substitutable methods, materials and the like are provided. Recital of one or a few synonyms or equivalents does not exclude use of other synonyms or equivalents, unless it is explicitly stated. Use of examples, including examples of terms, is for illustrative purposes only and does not limit the scope and meaning of the aspects of the invention herein.

The term "subject," refers to an individual organism such as a human or an animal. In embodiments, the subject is a mammal (*e.g.,* a human, a non-human primate, or a non-human mammal), a vertebrate, a laboratory animal, a domesticated animal, an agricultural animal, or a companion animal. In embodiments, the subject is a human (*e.g.,* a human patient). In embodiments, the subject is a rodent, a mouse, a rat, a hamster, a rabbit, a dog, a cat, a cow, a goat, a sheep, or a pig.

As used in this Specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive and covers both "or" and "and". Likewise, the term "and/or" covers both "or" and "and".

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About is

understood to be within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from the context, all numerical values provided herein are modified by the term "about."

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Evaluation of multivalent multispecific constructs

Construct design

In a non-limiting example, a multivalent multispecific construct is made having one or more of the following structures.

- X-IgG format:
    - a therapeutic payload; and
    - two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, optionally a second L3, a second ABD A, a second L2, a second ABD B, a second L1, and a second ABD C; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C; and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.
- Y-IgG format:
    - a therapeutic payload; and
    - two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD

B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

- Pronged format:
    - a therapeutic payload; and
    - four antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region; wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain.
- Ring format:
    - a therapeutic payload; and
    - ‘n’ antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, and a fourth linker (L4); wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 optionally attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4 or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4; wherein the scaffold region comprises a rigid polypeptide; wherein the construct further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring; and wherein n is 3-12.

In a non-limiting example, each of ABD A, ABD B, and ABD C independently binds to one of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, Her2, EGFR, PDL1, CEA, FolRa, DR5, mesothelin, CEACAM5, CD38, CD19, ALPL2, PRLR, Integrin, CD20, FcRn, FcRH5, GPRC5D, and CD79b.

Evaluation of the constructs

In a non-limiting example, the constructs are evaluated for targeted delivery and efficacy of the therapeutic payload using methods known in the art. Examples of assays that can be used to evaluate the constructs include, but are not limited to, assays for evaluating therapeutic payload delivery (e.g., specificity of binding to target cells, affinity for target cells, internalization efficacy, etc.) and therapeutic payload efficacy (e.g., depending on the payload, levels of cell killing, cytotoxicity, apoptosis induction, cell stimulation, gene editing, RNAi silencing, and mRNA expression, etc.). The constructs can then be evaluated for their efficiency in targeted delivery of therapeutic payloads using animal models.

Example 2: Experimental evaluation of multivalent multispecific constructs

Flow binding assay

Cells and primary antibodies of interest were prepared in cell culture media with 10% heat-inactivated fetal bovine serum and plated together in 96-well V-bottom plates (ThermoFisher #277143). Cells were incubated with antibodies in dark at 4°C for 30 minutes. Plates were washed with Staining Buffer (Invitrogen #00-4222-26) twice before resuspended in staining cocktail containing fixable viability dye and fluorescent secondary antibody at manufacturer's recommended concentrations for an incubation time of 30 minutes at 4°C in dark. Plates were washed once with Staining Buffer, and cells were then resuspended in Staining Buffer before flow cytometry analysis on the BioRad ZE5 Analyzer. Flow cytometry data was analyzed using FCS Express software and the median fluorescent intensity values were exported and plotted using Graphpad Prism software.

OX40 and 41BB activation reporter assay

OX40 and 41BB activation were evaluated using Promega's luciferase reporter systems (catalog number JA2195 and JA2351). Briefly, genetically engineered reporter Jurkat cells were incubated with specified concentrations of test articles in RPMI 1640 media with 10% heat-inactivated fetal bovine serum on 384-well opaque assay plates (ThermoFisher #164610) for 6 hours at 37°C with 5% CO_2. Plates were then removed from the incubators and equilibrated to room temperature before luminescence readout using Bio-glo substrate (Promega, catalog number G7940) according to manufacturer's protocol.

Antigen quantitation assay

Fluorescent antibodies against antigens of interest and corresponding isotype controls were prepared at saturating concentrations in 1X pH 7.4 phosphate buffered saline containing fixable viability dye. Cells and quantitation beads (Bangs Laboratories #815) were incubated with prepared fluorescent antibodies at 4°C for 30 minutes in dark. Assay plates were washed once with staining buffer (Invitrogen #00-4222-26) and cells were resuspended in staining buffer for flow analysis on the BioRad ZE5 Analyzer. Specific antibody binding capacities (ABC) on cell lines were determined using regression analysis template provided by Bangs Laboratories (QuickCal® v. 3.0 Data Analysis Template).

Internalization assay using Alexa Fluor 488 quenching method

Cells were prepared in cell culture media with 10% heat-inactivated fetal bovine serum and plated on 96-well V-bottom plates (ThermoFisher #277143). Antibodies of interest were pre-incubated with Alexa Fluor 488 secondary antibody in media at room temperature for at least 10 minutes (1:1 molar ratio of primary:AF488 Mouse Anti-Human IgG Fc [Southern Biotech #9040-30] or 1:2 molar ratio of primary:alpaca anti-human IgG, recombinant VHH, CoraLite® Plus 488 [Proteintech Group #shuGCL488-2]). The primary:secondary mixture was then added to plated cells for 4h, 1h, 30 min, and 15 min incubation times at 37°C or 4°C in the dark. All plates were chilled on ice for 10 minutes after incubation and kept chilled for all subsequent steps. Plates were washed with Staining Buffer and resuspended in fixable viability dye following manufacturer recommendations. Every internalization condition was split to a fresh plate for quenching (each well was resuspended in 50 uL viability dye stain and 25 uL were split into the fresh plate). Plates were incubated away from light at 4°C for 15 minutes, then washed again with Staining Buffer. Cells were resuspended either in Staining Buffer or Quenching Antibody (Alexa Fluor 488 Polyclonal Antibody, ThermoFisher #A11094) prior to flow readout on the BioRad ZE5 Analyzer (temperature controlled to 4°C prior and during readout). Internalization of antibodies was determined by the median fluorescence intensity of the Alexa Fluor 488 channel.

ADC Cytotoxicity Assay

Drug-conjugated secondary antibodies (MORADEC #AH-202AE-50, MORADEC #AH-207DX-50) were pre-incubated with primary antibodies of interest in RPMI 1640 media with 10% heat-inactivated fetal bovine serum. Titrations of this mixture was made by diluting into media and incubating at room temperature for 10 minutes. NCI-H929 cells (ATCC CRL-3580) and other cells of interest were prepared at 0.2 million/mL in culture media and 25 uL (5k) cells were distributed to each well of a 384-well culture plate (ThermoFisher #164610). Equal volumes of primary:secondary-drug mix was aliquoted to cells for a total well volume of 50 uL. Assay plates were incubated in a cell culture incubator (37°C, 5% CO_2) for 72 hours. Cell Titer Glo (CTG

2.0, Promega #G9242) was equilibrated to room temperature before adding to room temperature assay plates. A 1:2 ratio of CTG:well volume was added to each assay well and incubated at room temperature for 10-15 minutes in the dark. Cell viability was evaluated through luminescent detection of ATP on Promega GloMax.

GIP/GLP Activation Assay with Reporter Cell Line

Genetically engineered 293T-CRE-Luc2-GLP1R-T2A-GIPR reporter cells (KYinno KC- 3142) and antibodies of interest were prepared in DMEM media with 10% heat-inactivated fetal bovine serum. Cells were plated at 10k/well in 384-well opaque assay plates (ThermoFisher #164610) and allowed to attach overnight in an incubator (37°C, 5% CO_2). Antibody titrations were prepared and added to assay plates after the overnight seeding and allowed to incubate together for 5-6 hours (37°C, 5% CO_2). Following manufacturer's protocols, Bio-Glo Luciferase Assay Reagent was prepared and equilibrated to room temperature before adding to assay plates at a 1:2 volume ratio. Plates were incubated away from light for 10 minutes before luminescence readout. Cell activation was evaluated through luminescence on Promega GloMax.

Site-specific labeling of antibodies

Fluorescent dyes such as Alexa Fluor 488 and pHrodo Red were conjugated to the glycans on the Fc region of IgG antibodies, VHH-Fc or other Fc fusion proteins using Invitrogen SiteClick Antibody Labeling Kits (SiteClick™ Antibody Azido Modification Kit, cat# S10901; SiteClick™ Alexa Fluor™ 488 sDIBO Alkyne, cat# S10904; SiteClick™ pHrodo™ iFL Red sDIBO Alkyne, cat# S10903) according to manufacturer's protocol with minor adaptations based on the scale of the labeling reactions. Briefly, antibodies or Fc fusion proteins were first concentrated using Pierce Protein Concentrators (Thermo Scientific, cat# 88513) and then buffer exchanged into 1X TBS buffer (pH 7.2-7.5) using Zeba desalting columns (Thermo Scientific, cat# A57759). The protein samples were then incubated with β-galactosidase overnight at 37 °C to remove terminal galactose residues on the glycans. Next day, UDP-GalNAz, GalT enzyme, Tris buffer and kit supplied buffer additive were added to the protein samples and let the reaction go overnight at 30 °C. The samples were then purified with Zeba desalting columns before mixed with the sDIBO alkyne conjugation reagents for overnight incubation at room temperature. Excess dyes were removed with Zeba desalting columns or dye removal columns and the degree of labeling were determined based on measured absorbance at specific wavelengths according to the SiteClick kit manual.

Figures 5-15 depict results characterizing multivalent and multivalent-multispecific constructs described herein using these methods.

As shown in **Figures 5A and 5B**, increasing multivalency enhanced binding and functional activation. The data demonstrate that multivalent constructs described herein having four (Y4 format) or six ABDs (Y6 format) have increased binding (**Figure 5A**) and functional activity (**Figure 5B**) when compared to constructs only having only two ABDs (Y2, similar to IgG's).

As shown in **Figure 6A and B**, multivalent multispecific constructs can be produced at high titer and purity. As described herein constructs having 12 ABDs (X12 format) have increased activity when compared to constructs having only two or six ABDs, and multivalent multispecific constructs described herein comprising an OX40 ABD, PDL-1 ABD, and 4-1BB ABD, demonstrate increased activity in an OX40 reporter assay when the OX40 ABD is present at an external position.

As shown in **Figure 7**, mixed multivalent multispecific constructs described herein having two 41BB ligand peptide trimers and two, four or six CD38 VHHs demonstrated further increased activity compared to the constructs evaluated in Figure 6.

As shown in **Figures 8A-8C**, inclusion of a second hinge in a multivalent multispecific construct described herein allowed conjugation of four additional cysteines upon limited reduction of interchain disulfides, and incorporation of C-terminal hinges of a variety of sequences in a multivalent format has no negative impact on activity in the OX40 activation assay, indicating proper expression and assembly.

As shown in **Figures 9-11**, multivalent constructs described herein having four or six ABDs showed log order increases in internalization when compared to constructs having only two ABDs, and the increases depended on target and antigen density on the target cell. The figures show the results of internalization assays on constructs comprising BCMA ABDs (**Figure 9**); GPRC5D (**Figure 10**), and CD38 (**Figure 11**).

As shown in **Figure 12**, triple targeting BCMA/CD38/GPRC5D Y6 constructs showed log order better internalization than Y2 constructs.

As shown in **Figure 13**, a triple targeting BCMA/CD38/GPRC5D Y6 construct showed an advantage compared to the Y2 and IgG like controls in a secondary antibody based cell killing assay.

In summary, the multivalent multispecific constructs described herein show large (>10X) antigen-, epitope- and antigen density-dependent increases in binding, function, internalization and killing.

Example 3: Evaluation of multivalent multispecific GLP-1-like peptide constructs

GLP-1 like peptides and derivatives thereof have been used very successfully to treat diabetes and obesity. Incretin peptides interact with GPCR's to cause downstream signaling in relevant cells. Because of the close interaction between peptide and cognate GPCR – fusion of peptides to half-life extending features like Fc domains from IgG's typically results in significant loss of activity relative to native incretin. Multivalent

constructs in the format described herein were evaluated for their ability to show activity in functional assays on relevant reporter cell lines.

As shown in **Figure 14**, Y6 formats of a GLP-1-like peptide were able to recover the activity lost by fusion and showed comparable activity to free GLP-1 control.

Example 4: Evaluation of multivalent multispecific X12 vs Y6

As shown in **Figure 15**, internalization of X12 based constructs is superior to Y6 on a OX40 expressing cell line.

INCORPORATION BY REFERENCE

All patents and publications referenced herein are hereby incorporated by reference in their entireties.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

As used herein, all headings are simply for organization and are not intended to limit the disclosure in any manner. The content of any individual section may be equally applicable to all sections.

EMBODIMENTS

Various additional embodiments of the disclosure are provided by the following enumerated embodiments, which can be combined in any number and in any combination.

Embodiment 1. A multivalent multispecific construct comprising two or more antigen-binding polypeptides and a conjugated therapeutic payload, wherein each of the two or more antigen-binding polypeptides comprises three or more antigen-binding domains, wherein each of the three or more antigen-binding domains of each of the two or more antigen-binding polypeptides can bind its respective target on a target cell, wherein the construct can bind to four or more targets on the surface of the target cell contemporaneously.

Embodiment 2. The multivalent multispecific construct of Embodiment 1, wherein the construct comprises:

(a) a therapeutic payload; and

(b) two or more antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-

binding domain (ABD B), a second linker (L2), a third antigen binding domain (ABD A), optionally a third linker (L3), and a scaffold region;

wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and

wherein the scaffold region comprises a ring, a central protein anchor, or an IgG Fc, or a portion of or a modified form thereof.

Embodiment 3. The multivalent multispecific construct of Embodiment 2, wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, and wherein the construct comprises two of the antigen-binding polypeptides.

Embodiment 4. The multivalent multispecific construct of Embodiment 3, wherein each of the two antigen-binding polypeptides further comprises, arranged from N- to C-terminus, (i) ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C, or (ii) L3 – ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C.

Embodiment 5. The multivalent multispecific construct of Embodiment 2, wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain, and wherein the construct comprises four of the antigen-binding polypeptides.

Embodiment 6. The multivalent multispecific construct of Embodiment 2, wherein the scaffold region comprises a rigid polypeptide; wherein the construct comprises ‘n’ of the antigen-binding polypeptides; wherein each of the antigen-binding polypeptides further comprises a fourth linker (L4), wherein L4 attaches the scaffold region to ABD C; and wherein the scaffold region further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring that forms a closed ring, wherein n is 3-12.

Embodiment 7. The multivalent multispecific construct of any one of Embodiments 2-6, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

Embodiment 8. The multivalent multispecific construct of any one of Embodiments 2-7, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

Embodiment 9. The multivalent multispecific construct of any one of Embodiments 2-8, wherein ABD C, ABD B, and/or ABD A targets a specific cell type.

Embodiment 10. The multivalent multispecific construct of any one of Embodiments 2-9, wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

Embodiment 11. The multivalent multispecific construct of any one of Embodiments 2-10, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell, optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

Embodiment 12. The multivalent multispecific construct of any one of Embodiments 2-11, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell.

Embodiment 13. The multivalent multispecific construct of any one of Embodiments 2-12, wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

Embodiment 14. The multivalent multispecific construct of any one of Embodiments 2-13, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

Embodiment 15. The multivalent multispecific construct of any one of Embodiments 2-14, wherein ABD C, ABD B, and/or ABD A comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue.

Embodiment 16. The multivalent multispecific construct of any one of Embodiments 2-15, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule.

Embodiment 17. The multivalent multispecific construct of any one of Embodiments 2-16, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

Embodiment 18. The multivalent multispecific construct of any one of Embodiments 2-17, wherein ABD C serves as a primary anchor for binding to a target cell.

Embodiment 19. The multivalent multispecific construct of any one of Embodiments 2-18, wherein ABD B only substantially stably engages with its target when ABD C is bound to its target and/or ABD A is bound to its target.

Embodiment 20. The multivalent multispecific construct of any one of Embodiments 2-19, wherein ABD A serves as a secondary anchor for binding to a target cell.

Embodiment 21. The multivalent multispecific construct of any one of Embodiments 2-20, wherein ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, and Her2.

Embodiment 22. The multivalent multispecific construct of any one of Embodiments 2-21, wherein ABD B binds one or more of EGFR, PDL1, CEA, FolRa, DR5, MUC1, mesothelin, Claudin18.2, CEACAM5, CD38, and CD19.

Embodiment 23. The multivalent multispecific construct of any one of Embodiments 2-22, wherein ABD A binds one or more of ALPL2, PRLR, Integrin, DR5, CD20, FcRn, EGFR, Trop2, Claudin18.2, FcRH5, and CD79b.

Embodiment 24. The multivalent multispecific construct of any one of Embodiments 2-23, wherein one of ABD C, ABD B, and ABD A binds Her2, and wherein one of ABD C, ABD B, and ABD A binds Trop2.

Embodiment 25. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 26. The multivalent multispecific construct of Embodiment 25, wherein the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

Embodiment 27. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 28. The multivalent multispecific construct of Embodiment 27, wherein the multivalent multispecific construct is suitable for treatment of NSCLC.

Embodiment 29. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 30. The multivalent multispecific construct of Embodiment 29, wherein the multivalent multispecific construct is suitable for treatment of GI cancer.

Embodiment 31. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 32. The multivalent multispecific construct of Embodiment 31, wherein the multivalent multispecific construct is suitable for treatment of myeloma.

Embodiment 33. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 34. The multivalent multispecific construct of Embodiment 33, wherein the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

Embodiment 35. The multivalent multispecific construct of any one of Embodiments 2-24, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 36. The multivalent multispecific construct of Embodiment 35, wherein the multivalent multispecific construct is suitable for treatment of breast cancer.

Embodiment 37. The multivalent multispecific construct of any one of Embodiments 2-36, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

Embodiment 38. The multivalent multispecific construct of any one of Embodiments 2-37, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

Embodiment 39. The multivalent multispecific construct of any one of Embodiments 2-38, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

Embodiment 40. The multivalent multispecific construct of Embodiment 39, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, (Glu-

Ala-Ala-Ala-Lys)$_{1-3}$ (SEQ ID NO: 13), (Ala-Pro)$_n$ (SEQ ID NO: 14), IgG C_H1, IgG C_H2-C_H3, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 41. The multivalent multispecific construct of any one of Embodiments 2-40, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6.

Embodiment 42. The multivalent multispecific construct of any one of Embodiments 2-41, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.5 to about 0.7, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6 to about 0.65.

Embodiment 43. The multivalent multispecific construct of any one of Embodiments 2-42, wherein L1, L2, L3, and/or L4 is substantially nonimmunogenic.

Embodiment 44. The multivalent multispecific construct of any one of Embodiments 2-43, wherein L1, L2, L3, and/or L4 is substantially non-cleavable.

Embodiment 45. The multivalent multispecific construct of any one of Embodiments 2-44, wherein L1, L2, L3, and/or L4 is substantially cleavable.

Embodiment 46. The multivalent multispecific construct of any one of Embodiments 2-45, wherein L1, L2, L3, and/or L4 is about 5-10 nm long; optionally about 7 nm long.

Embodiment 47. The multivalent multispecific construct of any one of Embodiments 2-46, wherein L3 is a rigid linker, is about 7-12 nm long, and/or comprises an alpha helix.

Embodiment 48. The multivalent multispecific construct of any one of Embodiments 2-47, wherein L2 is a moderately rigid linker, is about 7-12 nm long, and/or comprises b2-microglobulin, Zn-a2-glycoprotein, tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity, optionally wherein L2 is a cleavable linker.

Embodiment 49. The multivalent multispecific construct of any one of Embodiments 2-48, wherein L1 is a flexible linker, is about 7-12 nm long, and/or (Gly-Ser)$_n$, (Gly$_4$-Ser)$_n$ (SEQ ID NO: 1), (Gly$_3$-Ser)$_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), (Gly$_4$-Ser)$_3$, (SEQ ID NO: 10), (Gly$_2$-Ser)$_3$ (SEQ ID NO: 11), (Gly$_2$-Ser)$_2$ (SEQ ID

NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG C_H2-C_H3, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 50. The multivalent multispecific construct of any one of Embodiments 2-49, wherein the linkers, in order from most to least rigid, are L4, L3, L2, and L1.

Embodiment 51. The multivalent multispecific construct of any one of Embodiments 2-50, wherein the scaffold region comprises a rigid protein.

Embodiment 52. The multivalent multispecific construct of any one of Embodiments 2-51, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG C_H2-C_H3, human serum albumin, human transferrin, or a fragment thereof.

Embodiment 53. The multivalent multispecific construct of any one of Embodiments 2-52, wherein the scaffold region comprises IgG C_H2-C_H3, or a fragment thereof, optionally wherein the IgG C_H2-C_H3 further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-C_H2-C_H3-hinge.

Embodiment 54. The multivalent multispecific construct of any one of Embodiments 2-53, wherein the payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region.

Embodiment 55. The multivalent multispecific construct of any one of Embodiments 2-54, wherein the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

Embodiment 56. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload comprises a protein, optionally wherein the protein comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

Embodiment 57. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload comprises a small molecule, optionally wherein the small molecules comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist.

Embodiment 58. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload comprises an RNA molecule, optionally wherein the RNA molecule is an siRNA molecule.

Embodiment 59. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload comprises a chemosensitizer.

Embodiment 60. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload comprises a genetic modifier.

Embodiment 61. The multivalent multispecific construct of any one of Embodiments 2-55, wherein the payload is a belotecan derivative attached to the scaffold region with a cathepsin cleavable linker.

Embodiment 62. The multivalent multispecific construct of any one of Embodiments 2-61, wherein the multivalent multispecific construct comprises more than one payload, optionally wherein the drug to antibody ratio (DAR) is about 2-8.

Embodiment 63. The multivalent multispecific construct of any one of Embodiments 2-62, wherein the multivalent multispecific construct further comprises one or more enhancer domains and/or one or more FcRn binding domain, optionally wherein the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

Embodiment 64. The multivalent multispecific construct of any one of Embodiments 2-63, wherein the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm.

Embodiment 65. The multivalent multispecific construct of any one of Embodiments 2-64, wherein each antigen-binding polypeptide contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Embodiment 66. The multivalent multispecific construct of any one of Embodiments 2-65, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Embodiment 67. A nucleic acid encoding the multivalent multispecific construct of any one of Embodiments 1-66,.

Embodiment 68. A host cell comprising the nucleic acid of Embodiment 67.

Embodiment 69. A pharmaceutical composition comprising the multivalent multispecific construct of any one of Embodiments 1-66, the nucleic acid of Embodiment 67, or the host cell of Embodiment 68.

Embodiment 70. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 1-66, the nucleic acid of Embodiment 67, the host cell of Embodiment 68, or the pharmaceutical composition of Embodiment 69.

Embodiment 71. The method of Embodiment 70, wherein the cancer is a solid tumor.

Embodiment 72. The method of Embodiment 71, wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

Embodiment 73. The method of Embodiment 70, wherein the cancer is a hematological cancer.

Embodiment 74. The method of Embodiment 73, wherein the cancer is leukemia, lymphoma, or multiple myeloma.

Embodiment 75. A method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 76. A method of treating NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 77. A method of treating GI cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 78. A method of treating myeloma in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 79. A method of treating NHL or autoimmunity in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 80. A method of treating breast cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 2-69, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 81. A multivalent multispecific construct comprising:

(a) a therapeutic payload; and

(b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, optionally a second L3, a second ABD A, a second L2, a second ABD B, a second L1, and a second ABD C;

wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C; and

wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

Embodiment 82. The multivalent multispecific construct of Embodiment 81, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

Embodiment 83. The multivalent multispecific construct of any one of Embodiments 81-82, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

Embodiment 84. The multivalent multispecific construct of any one of Embodiments 81-83, wherein ABD C, ABD B, and/or ABD A targets a specific cell type.

Embodiment 85. The multivalent multispecific construct of any one of Embodiments 81-84, wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

Embodiment 86. The multivalent multispecific construct of any one of Embodiments 81-85, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell,

optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

Embodiment 87. The multivalent multispecific construct of any one of Embodiments 81-86, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell.

Embodiment 88. The multivalent multispecific construct of any one of Embodiments 81-87, wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

Embodiment 89. The multivalent multispecific construct of any one of Embodiments 81-88, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

Embodiment 90. The multivalent multispecific construct of any one of Embodiments 81-89, wherein ABD C, ABD B, and/or ABD A comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue.

Embodiment 91. The multivalent multispecific construct of any one of Embodiments 81-90, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule.

Embodiment 92. The multivalent multispecific construct of any one of Embodiments 81-91, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

Embodiment 93. The multivalent multispecific construct of any one of Embodiments 81-92, wherein ABD C serves as a primary anchor for binding to a target cell.

Embodiment 94. The multivalent multispecific construct of any one of Embodiments 81-93, wherein ABD B only substantially stably engages with its target when ABD C is bound to its target and/or ABD A is bound to its target.

Embodiment 95. The multivalent multispecific construct of any one of Embodiments 81-94, wherein ABD A serves as a secondary anchor for binding to a target cell.

Embodiment 96. The multivalent multispecific construct of any one of Embodiments 81-95, wherein ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, and Her2.

Embodiment 97. The multivalent multispecific construct of any one of Embodiments 81-96, wherein ABD B binds one or more of EGFR, PDL1, CEA, FolRa, DR5, MUC1, mesothelin, Claudin18.2, CEACAM5, CD38, and CD19.

Embodiment 98. The multivalent multispecific construct of any one of Embodiments 81-97, wherein ABD A binds one or more of ALPL2, PRLR, Integrin, DR5, CD20, FcRn, EGFR, Trop2, Claudin18.2, FcRH5, and CD79b.

Embodiment 99. The multivalent multispecific construct of any one of Embodiments 81-98, wherein one of ABD C, ABD B, and ABD A binds Her2, and wherein one of ABD C, ABD B, and ABD A binds Trop2.

Embodiment 100. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 101. The multivalent multispecific construct of Embodiment 100, wherein the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

Embodiment 102. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 103. The multivalent multispecific construct of Embodiment 102, wherein the multivalent multispecific construct is suitable for treatment of NSCLC.

Embodiment 104. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 105. The multivalent multispecific construct of Embodiment 104, wherein the multivalent multispecific construct is suitable for treatment of GI cancer.

Embodiment 106. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 107. The multivalent multispecific construct of Embodiment 106, wherein the multivalent multispecific construct is suitable for treatment of myeloma.

Embodiment 108. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 109. The multivalent multispecific construct of Embodiment 108, wherein the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

Embodiment 110. The multivalent multispecific construct of any one of Embodiments 81-99, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 111. The multivalent multispecific construct of Embodiment 110, wherein the multivalent multispecific construct is suitable for treatment of breast cancer.

Embodiment 112. The multivalent multispecific construct of any one of Embodiments 81-111, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

Embodiment 113. The multivalent multispecific construct of any one of Embodiments 81-112, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

Embodiment 114. The multivalent multispecific construct of any one of Embodiments 81-113, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

Embodiment 115. The multivalent multispecific construct of Embodiment 114, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 116. The multivalent multispecific construct of any one of Embodiments 81-115, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6.

Embodiment 117. The multivalent multispecific construct of any one of Embodiments 81-116, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.5 to about 0.7, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6 to about 0.65.

Embodiment 118. The multivalent multispecific construct of any one of Embodiments 81-117, wherein L1, L2, L3, and/or L4 is substantially nonimmunogenic.

Embodiment 119. The multivalent multispecific construct of any one of Embodiments 81-118, wherein L1, L2, L3, and/or L4 is substantially non-cleavable.

Embodiment 120. The multivalent multispecific construct of any one of Embodiments 81-119, wherein L1, L2, L3, and/or L4 is substantially cleavable.

Embodiment 121. The multivalent multispecific construct of any one of Embodiments 81-120, wherein L1, L2, L3, and/or L4 is about 5-10 nm long; optionally about 7 nm long.

Embodiment 122. The multivalent multispecific construct of any one of Embodiments 81-121, wherein L3 is a rigid linker, is about 7-12 nm long, and/or comprises an alpha helix.

Embodiment 123. The multivalent multispecific construct of any one of Embodiments 81-122, wherein L2 is a moderately rigid linker, is about 7-12 nm long, and/or comprises b2-microglobulin, Zn-a2-glycoprotein, tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity, optionally wherein L2 is a cleavable linker.

Embodiment 124. The multivalent multispecific construct of any one of Embodiments 81-123, wherein L1 is a flexible linker, is about 7-12 nm long, and/or comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 125. The multivalent multispecific construct of any one of Embodiments 81-124, wherein the linkers, in order from most to least rigid, are L4, L3, L2, and L1.

Embodiment 126. The multivalent multispecific construct of any one of Embodiments 81-125, wherein the scaffold region comprises a rigid protein.

Embodiment 127. The multivalent multispecific construct of any one of Embodiments 81-126, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG $C_H2\text{-}C_H3$, human serum albumin, human transferrin, or a fragment thereof.

Embodiment 128. The multivalent multispecific construct of any one of Embodiments 81-127, wherein the scaffold region comprises IgG $C_H2\text{-}C_H3$, or a fragment thereof, optionally wherein the IgG $C_H2\text{-}C_H3$ further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-$C_H2\text{-}C_H3$-hinge.

Embodiment 129. The multivalent multispecific construct of any one of Embodiments 81-128, wherein the payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region.

Embodiment 130. The multivalent multispecific construct of any one of Embodiments 81-129, wherein the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

Embodiment 131. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload comprises a protein, optionally wherein the protein comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

Embodiment 132. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload comprises a small molecule, optionally wherein the small molecules comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist.

Embodiment 133. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload comprises an RNA molecule, optionally wherein the RNA molecule is an siRNA molecule.

Embodiment 134. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload comprises a chemosensitizer.

Embodiment 135. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload comprises a genetic modifier.

Embodiment 136. The multivalent multispecific construct of any one of Embodiments 81-130, wherein the payload is a belotecan derivative attached to the scaffold region with a cathepsin cleavable linker.

Embodiment 137. The multivalent multispecific construct of any one of Embodiments 81-136, wherein the multivalent multispecific construct comprises more than one payload, optionally wherein the drug to antibody ratio (DAR) is about 2-8.

Embodiment 138. The multivalent multispecific construct of any one of Embodiments 81-137, wherein the multivalent multispecific construct further comprises one or more enhancer domains and/or one or more FcRn binding domain, optionally wherein the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

Embodiment 139. The multivalent multispecific construct of any one of Embodiments 81-138, wherein the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm.

Embodiment 140. The multivalent multispecific construct of any one of Embodiments 81-139, wherein each antigen-binding polypeptide contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Embodiment 141. The multivalent multispecific construct of any one of Embodiments 81-140, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Embodiment 142. A nucleic acid encoding the multivalent multispecific construct of any one of Embodiments 81-141.

Embodiment 143. A host cell comprising the nucleic acid of Embodiment 142.

Embodiment 144. A pharmaceutical composition comprising the multivalent multispecific construct of any one of Embodiments 81-141, the nucleic acid of Embodiment 142, or the host cell of Embodiment 143.

Embodiment 145. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-141, the nucleic acid of Embodiment 142, the host cell of Embodiment 143, or the pharmaceutical composition of Embodiment 144.

Embodiment 146. The method of Embodiment 145, wherein the cancer is a solid tumor.

Embodiment 147. The method of Embodiment 146, wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

Embodiment 148. The method of Embodiment 145, wherein the cancer is a hematological cancer.

Embodiment 149. The method of Embodiment 148, wherein the cancer is leukemia, lymphoma, or multiple myeloma.

Embodiment 150. A method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 151. A method of treating NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 152. A method of treating GI cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 153. A method of treating myeloma in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 154. A method of treating NHL or autoimmunity in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 155. A method of treating breast cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 81-144, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 156. A multivalent multispecific construct comprising:

(a) a therapeutic payload; and

(b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region;

wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and

wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

Embodiment 157. The multivalent multispecific construct of Embodiment 156, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

Embodiment 158. The multivalent multispecific construct of any one of Embodiments 156-157, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

Embodiment 159. The multivalent multispecific construct of any one of Embodiments 156-158, wherein ABD C, ABD B, and/or ABD A targets a specific cell type.

Embodiment 160. The multivalent multispecific construct of any one of Embodiments 156-159, wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

Embodiment 161. The multivalent multispecific construct of any one of Embodiments 156-160, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell, optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

Embodiment 162. The multivalent multispecific construct of any one of Embodiments 156-161, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell.

Embodiment 163. The multivalent multispecific construct of any one of Embodiments 156-162, wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

Embodiment 164. The multivalent multispecific construct of any one of Embodiments 156-163, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

Embodiment 165. The multivalent multispecific construct of any one of Embodiments 156-164, wherein ABD C, ABD B, and/or ABD A comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue.

Embodiment 166. The multivalent multispecific construct of any one of Embodiments 156-165, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule.

Embodiment 167. The multivalent multispecific construct of any one of Embodiments 156-166, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

Embodiment 168. The multivalent multispecific construct of any one of Embodiments 156-167, wherein ABD C serves as a primary anchor for binding to a target cell.

Embodiment 169. The multivalent multispecific construct of any one of Embodiments 156-168, wherein ABD B only substantially stably engages with its target when ABD C is bound to its target and/or ABD A is bound to its target.

Embodiment 170. The multivalent multispecific construct of any one of Embodiments 156-169, wherein ABD A serves as a secondary anchor for binding to a target cell.

Embodiment 171. The multivalent multispecific construct of any one of Embodiments 156-170, wherein ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, and Her2.

Embodiment 172. The multivalent multispecific construct of any one of Embodiments 156-171, wherein ABD B binds one or more of EGFR, PDL1, CEA, FolRa, DR5, MUC1, mesothelin, Claudin18.2, CEACAM5, CD38, and CD19.

Embodiment 173. The multivalent multispecific construct of any one of Embodiments 156-172, wherein ABD A binds one or more of ALPL2, PRLR, Integrin, DR5, CD20, FcRn, EGFR, Trop2, Claudin18.2, FcRH5, and CD79b.

Embodiment 174. The multivalent multispecific construct of any one of Embodiments 156-173, wherein one of ABD C, ABD B, and ABD A binds Her2, and wherein one of ABD C, ABD B, and ABD A binds Trop2.

Embodiment 175. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 176. The multivalent multispecific construct of Embodiment 175, wherein the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

Embodiment 177. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 178. The multivalent multispecific construct of Embodiment 177, wherein the multivalent multispecific construct is suitable for treatment of NSCLC.

Embodiment 179. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 180. The multivalent multispecific construct of Embodiment 179, wherein the multivalent multispecific construct is suitable for treatment of GI cancer.

Embodiment 181. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 182. The multivalent multispecific construct of Embodiment 181, wherein the multivalent multispecific construct is suitable for treatment of myeloma.

Embodiment 183. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 184. The multivalent multispecific construct of Embodiment 183, wherein the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

Embodiment 185. The multivalent multispecific construct of any one of Embodiments 156-174, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 186. The multivalent multispecific construct of Embodiment 185, wherein the multivalent multispecific construct is suitable for treatment of breast cancer.

Embodiment 187. The multivalent multispecific construct of any one of Embodiments 156-186, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

Embodiment 188. The multivalent multispecific construct of any one of Embodiments 156-187, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

Embodiment 189. The multivalent multispecific construct of any one of Embodiments 156-188, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

Embodiment 190. The multivalent multispecific construct of Embodiment 189, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}$

Ser)$_3$, (SEQ ID NO: 10), (Gly$_2$-Ser)$_3$ (SEQ ID NO: 11), (Gly$_2$-Ser)$_2$ (SEQ ID NO: 12), Ferritin E Helix, (Glu-Ala-Ala-Ala-Lys)$_{1-3}$ (SEQ ID NO: 13), (Ala-Pro)$_n$ (SEQ ID NO: 14), IgG C_H1, IgG C_H2-C_H3, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 191. The multivalent multispecific construct of any one of Embodiments 156-190, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6.

Embodiment 192. The multivalent multispecific construct of any one of Embodiments 156-191, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.5 to about 0.7, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6 to about 0.65.

Embodiment 193. The multivalent multispecific construct of any one of Embodiments 156-192, wherein L1, L2, L3, and/or L4 is substantially nonimmunogenic.

Embodiment 194. The multivalent multispecific construct of any one of Embodiments 156-193, wherein L1, L2, L3, and/or L4 is substantially non-cleavable.

Embodiment 195. The multivalent multispecific construct of any one of Embodiments 156-194, wherein L1, L2, L3, and/or L4 is substantially cleavable.

Embodiment 196. The multivalent multispecific construct of any one of Embodiments 156-195, wherein L1, L2, L3, and/or L4 is about 5-10 nm long; optionally about 7 nm long.

Embodiment 197. The multivalent multispecific construct of any one of Embodiments 156-196, wherein L3 is a rigid linker, is about 7-12 nm long, and/or comprises an alpha helix.

Embodiment 198. The multivalent multispecific construct of any one of Embodiments 156-197, wherein L2 is a moderately rigid linker, is about 7-12 nm long, and/or comprises b2-microglobulin, Zn-a2-glycoprotein, tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity, optionally wherein L2 is a cleavable linker.

Embodiment 199. The multivalent multispecific construct of any one of Embodiments 156-198, wherein L1 is a flexible linker, is about 7-12 nm long, and/or comprises (Gly-Ser)$_n$, (Gly$_4$-Ser)$_n$ (SEQ ID NO: 1), (Gly$_3$-Ser)$_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), (Gly$_4$-Ser)$_3$, (SEQ ID NO: 10), (Gly$_2$-Ser)$_3$ (SEQ ID NO:

11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 200. The multivalent multispecific construct of any one of Embodiments 156-199, wherein the linkers, in order from most to least rigid, are L4, L3, L2, and L1.

Embodiment 201. The multivalent multispecific construct of any one of Embodiments 156-200, wherein the scaffold region comprises a rigid protein.

Embodiment 202. The multivalent multispecific construct of any one of Embodiments 156-201, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG $C_H2\text{-}C_H3$, human serum albumin, human transferrin, or a fragment thereof.

Embodiment 203. The multivalent multispecific construct of any one of Embodiments 156-202, wherein the scaffold region comprises IgG $C_H2\text{-}C_H3$, or a fragment thereof, optionally wherein the IgG $C_H2\text{-}C_H3$ further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-$C_H2\text{-}C_H3$-hinge.

Embodiment 204. The multivalent multispecific construct of any one of Embodiments 156-203, wherein the payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region.

Embodiment 205. The multivalent multispecific construct of any one of Embodiments 156-204, wherein the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

Embodiment 206. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload comprises a protein, optionally wherein the protein comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

Embodiment 207. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload comprises a small molecule, optionally wherein the small molecules comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist.

Embodiment 208. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload comprises an RNA molecule, optionally wherein the RNA molecule is an siRNA molecule.

Embodiment 209. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload comprises a chemosensitizer.

Embodiment 210. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload comprises a genetic modifier.

Embodiment 211. The multivalent multispecific construct of any one of Embodiments 156-205, wherein the payload is a belotecan derivative attached to the scaffold region with a cathepsin cleavable linker.

Embodiment 212. The multivalent multispecific construct of any one of Embodiments 156-211, wherein the multivalent multispecific construct comprises more than one payload, optionally wherein the drug to antibody ratio (DAR) is about 2-8.

Embodiment 213. The multivalent multispecific construct of any one of Embodiments 156-212, wherein the multivalent multispecific construct further comprises one or more enhancer domains and/or one or more FcRn binding domain, optionally wherein the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

Embodiment 214. The multivalent multispecific construct of any one of Embodiments 156-213, wherein the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm.

Embodiment 215. The multivalent multispecific construct of any one of Embodiments 156-214, wherein each antigen-binding polypeptide contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Embodiment 216. The multivalent multispecific construct of any one of Embodiments 156-215, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Embodiment 217. A nucleic acid encoding the multivalent multispecific construct of any one of Embodiments 156-216.

Embodiment 218. A host cell comprising the nucleic acid of Embodiment 217.

Embodiment 219. A pharmaceutical composition comprising the multivalent multispecific construct of any one of Embodiments 156-216, the nucleic acid of Embodiment 217, or the host cell of Embodiment 218.

Embodiment 220. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-216, the nucleic acid of Embodiment 217, the host cell of Embodiment 218, or the pharmaceutical composition of Embodiment 219.

Embodiment 221. The method of Embodiment 220, wherein the cancer is a solid tumor.

Embodiment 222. The method of Embodiment 221, wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

Embodiment 223. The method of Embodiment 220, wherein the cancer is a hematological cancer.

Embodiment 224. The method of Embodiment 223, wherein the cancer is leukemia, lymphoma, or multiple myeloma.

Embodiment 225. A method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 226. A method of treating NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 227. A method of treating GI cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 228. A method of treating myeloma in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 229. A method of treating NHL or autoimmunity in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 230. A method of treating breast cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 156-219, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 231. A multispecific construct comprising:

(a) a therapeutic payload; and

(b) four antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region;

wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and

wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain.

Embodiment 232. The multivalent multispecific construct of Embodiment 231, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

Embodiment 233. The multivalent multispecific construct of any one of Embodiments 231-232, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

Embodiment 234. The multivalent multispecific construct of any one of Embodiments 231-233, wherein ABD C, ABD B, and/or ABD A targets a specific cell type.

Embodiment 235. The multivalent multispecific construct of any one of Embodiments 231-234, wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

Embodiment 236. The multivalent multispecific construct of any one of Embodiments 231-235, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell,

optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

Embodiment 237. The multivalent multispecific construct of any one of Embodiments 231-236, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell.

Embodiment 238. The multivalent multispecific construct of any one of Embodiments 231-237, wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

Embodiment 239. The multivalent multispecific construct of any one of Embodiments 231-238, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

Embodiment 240. The multivalent multispecific construct of any one of Embodiments 231-239, wherein ABD C, ABD B, and/or ABD A comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue.

Embodiment 241. The multivalent multispecific construct of any one of Embodiments 231-240, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule.

Embodiment 242. The multivalent multispecific construct of any one of Embodiments 231-241, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

Embodiment 243. The multivalent multispecific construct of any one of Embodiments 231-242, wherein ABD C serves as a primary anchor for binding to a target cell.

Embodiment 244. The multivalent multispecific construct of any one of Embodiments 231-243, wherein ABD B only substantially stably engages with its target when ABD C is bound to its target and/or ABD A is bound to its target.

Embodiment 245. The multivalent multispecific construct of any one of Embodiments 231-244, wherein ABD A serves as a secondary anchor for binding to a target cell.

Embodiment 246. The multivalent multispecific construct of any one of Embodiments 231-245, wherein ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, and Her2.

Embodiment 247. The multivalent multispecific construct of any one of Embodiments 231-246, wherein ABD B binds one or more of EGFR, PDL1, CEA, FolRa, DR5, MUC1, mesothelin, Claudin18.2, CEACAM5, CD38, and CD19.

Embodiment 248. The multivalent multispecific construct of any one of Embodiments 231-247, wherein ABD A binds one or more of ALPL2, PRLR, Integrin, DR5, CD20, FcRn, EGFR, Trop2, Claudin18.2, FcRH5, and CD79b.

Embodiment 249. The multivalent multispecific construct of any one of Embodiments 231-248, wherein one of ABD C, ABD B, and ABD A binds Her2, and wherein one of ABD C, ABD B, and ABD A binds Trop2.

Embodiment 250. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 251. The multivalent multispecific construct of Embodiment 250, wherein the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

Embodiment 252. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 253. The multivalent multispecific construct of Embodiment 252, wherein the multivalent multispecific construct is suitable for treatment of NSCLC.

Embodiment 254. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 255. The multivalent multispecific construct of Embodiment 254, wherein the multivalent multispecific construct is suitable for treatment of GI cancer.

Embodiment 256. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 257. The multivalent multispecific construct of Embodiment 256, wherein the multivalent multispecific construct is suitable for treatment of myeloma.

Embodiment 258. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 259. The multivalent multispecific construct of Embodiment 258, wherein the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

Embodiment 260. The multivalent multispecific construct of any one of Embodiments 231-249, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 261. The multivalent multispecific construct of Embodiment 260, wherein the multivalent multispecific construct is suitable for treatment of breast cancer.

Embodiment 262. The multivalent multispecific construct of any one of Embodiments 231-261, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

Embodiment 263. The multivalent multispecific construct of any one of Embodiments 231-262, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

Embodiment 264. The multivalent multispecific construct of any one of Embodiments 231-263, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

Embodiment 265. The multivalent multispecific construct of Embodiment 264, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 266. The multivalent multispecific construct of any one of Embodiments 231-265, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6.

Embodiment 267. The multivalent multispecific construct of any one of Embodiments 231-266, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.5 to about 0.7, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6 to about 0.65.

Embodiment 268. The multivalent multispecific construct of any one of Embodiments 231-267, wherein L1, L2, L3, and/or L4 is substantially nonimmunogenic.

Embodiment 269. The multivalent multispecific construct of any one of Embodiments 231-268, wherein L1, L2, L3, and/or L4 is substantially non-cleavable.

Embodiment 270. The multivalent multispecific construct of any one of Embodiments 231-269, wherein L1, L2, L3, and/or L4 is substantially cleavable.

Embodiment 271. The multivalent multispecific construct of any one of Embodiments 231-270, wherein L1, L2, L3, and/or L4 is about 5-10 nm long; optionally about 7 nm long.

Embodiment 272. The multivalent multispecific construct of any one of Embodiments 231-271, wherein L3 is a rigid linker, is about 7-12 nm long, and/or comprises an alpha helix.

Embodiment 273. The multivalent multispecific construct of any one of Embodiments 231-272, wherein L2 is a moderately rigid linker, is about 7-12 nm long, and/or comprises b2-microglobulin, Zn-a2-glycoprotein, tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity, optionally wherein L2 is a cleavable linker.

Embodiment 274. The multivalent multispecific construct of any one of Embodiments 231-273, wherein L1 is a flexible linker, is about 7-12 nm long, and/or comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 275. The multivalent multispecific construct of any one of Embodiments 231-274, wherein the linkers, in order from most to least rigid, are L4, L3, L2, and L1.

Embodiment 276. The multivalent multispecific construct of any one of Embodiments 231-275, wherein the scaffold region comprises a rigid protein.

Embodiment 277. The multivalent multispecific construct of any one of Embodiments 231-276, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG $C_H2\text{-}C_H3$, human serum albumin, human transferrin, or a fragment thereof.

Embodiment 278. The multivalent multispecific construct of any one of Embodiments 231-277, wherein the scaffold region comprises IgG $C_H2\text{-}C_H3$, or a fragment thereof, optionally wherein the IgG $C_H2\text{-}C_H3$ further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-$C_H2\text{-}C_H3$-hinge.

Embodiment 279. The multivalent multispecific construct of any one of Embodiments 231-278, wherein the payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region.

Embodiment 280. The multivalent multispecific construct of any one of Embodiments 231-279, wherein the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

Embodiment 281. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload comprises a protein, optionally wherein the protein comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

Embodiment 282. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload comprises a small molecule, optionally wherein the small molecules comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist.

Embodiment 283. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload comprises an RNA molecule, optionally wherein the RNA molecule is an siRNA molecule.

Embodiment 284. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload comprises a chemosensitizer.

Embodiment 285. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload comprises a genetic modifier.

Embodiment 286. The multivalent multispecific construct of any one of Embodiments 231-280, wherein the payload is a belotecan derivative attached to the scaffold region with a cathepsin cleavable linker.

Embodiment 287. The multivalent multispecific construct of any one of Embodiments 231-286, wherein the multivalent multispecific construct comprises more than one payload, optionally wherein the drug to antibody ratio (DAR) is about 2-8.

Embodiment 288. The multivalent multispecific construct of any one of Embodiments 231-287, wherein the multivalent multispecific construct further comprises one or more enhancer domains and/or one or more FcRn binding domain, optionally wherein the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

Embodiment 289. The multivalent multispecific construct of any one of Embodiments 231-288, wherein the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm.

Embodiment 290. The multivalent multispecific construct of any one of Embodiments 231-289, wherein each antigen-binding polypeptide contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Embodiment 291. The multivalent multispecific construct of any one of Embodiments 231-290, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Embodiment 292. A nucleic acid encoding the multivalent multispecific construct of any one of Embodiments 231-291.

Embodiment 293. A host cell comprising the nucleic acid of Embodiment 292.

Embodiment 294. A pharmaceutical composition comprising the multivalent multispecific construct of any one of Embodiments 231-291, the nucleic acid of Embodiment 292, or the host cell of Embodiment 293.

Embodiment 295. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-291, the nucleic acid of Embodiment 292, the host cell of Embodiment 293, or the pharmaceutical composition of Embodiment 294.

Embodiment 296. The method of Embodiment 295, wherein the cancer is a solid tumor.

Embodiment 297. The method of Embodiment 296, wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

Embodiment 298. The method of Embodiment 295, wherein the cancer is a hematological cancer.

Embodiment 299. The method of Embodiment 298, wherein the cancer is leukemia, lymphoma, or multiple myeloma.

Embodiment 300. A method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 301. A method of treating NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 302. A method of treating GI cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 303. A method of treating myeloma in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 304. A method of treating NHL or autoimmunity in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 305. A method of treating breast cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 231-294, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 306. A multispecific construct comprising:

(a) a therapeutic payload; and

(b) 'n' antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, and a fourth linker (L4);

wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 optionally attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4 or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4;

wherein the scaffold region comprises a rigid polypeptide;

wherein the construct further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring; and
wherein n is 3-12.

Embodiment 307. The multivalent multispecific construct of Embodiment 306, wherein n is 3, 4, 5, 6, 7, 8, 9, or 10.

Embodiment 308. The multivalent multispecific construct of any one of Embodiments 306-307, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

Embodiment 309. The multivalent multispecific construct of any one of Embodiments 306-308, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

Embodiment 310. The multivalent multispecific construct of any one of Embodiments 306-309, wherein ABD C, ABD B, and/or ABD A targets a specific cell type.

Embodiment 311. The multivalent multispecific construct of any one of Embodiments 306-310, wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

Embodiment 312. The multivalent multispecific construct of any one of Embodiments 306-311, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell, optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

Embodiment 313. The multivalent multispecific construct of any one of Embodiments 306-312, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell.

Embodiment 314. The multivalent multispecific construct of any one of Embodiments 306-313, wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

Embodiment 315. The multivalent multispecific construct of any one of Embodiments 306-314, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

Embodiment 316. The multivalent multispecific construct of any one of Embodiments 306-315, wherein ABD C, ABD B, and/or ABD A comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue.

Embodiment 317. The multivalent multispecific construct of any one of Embodiments 306-316, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule.

Embodiment 318. The multivalent multispecific construct of any one of Embodiments 306-317, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

Embodiment 319. The multivalent multispecific construct of any one of Embodiments 306-318, wherein ABD C serves as a primary anchor for binding to a target cell.

Embodiment 320. The multivalent multispecific construct of any one of Embodiments 306-319, wherein ABD B only substantially stably engages with its target when ABD C is bound to its target and/or ABD A is bound to its target.

Embodiment 321. The multivalent multispecific construct of any one of Embodiments 306-320, wherein ABD A serves as a secondary anchor for binding to a target cell.

Embodiment 322. The multivalent multispecific construct of any one of Embodiments 306-321, wherein ABD C binds one or more of NaPi2b, Trop2, MUC1, Ly6G6D, Claudin18.2, B7H3, BCMA, CD22, and Her2.

Embodiment 323. The multivalent multispecific construct of any one of Embodiments 306-322, wherein ABD B binds one or more of EGFR, PDL1, CEA, FolRa, DR5, MUC1, mesothelin, Claudin18.2, CEACAM5, CD38, and CD19.

Embodiment 324. The multivalent multispecific construct of any one of Embodiments 306-323, wherein ABD A binds one or more of ALPL2, PRLR, Integrin, DR5, CD20, FcRn, EGFR, Trop2, Claudin18.2, FcRH5, and CD79b.

Embodiment 325. The multivalent multispecific construct of any one of Embodiments 306-324, wherein one of ABD C, ABD B, and ABD A binds Her2, and wherein one of ABD C, ABD B, and ABD A binds Trop2.

Embodiment 326. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 327. The multivalent multispecific construct of Embodiment 326, wherein the multivalent multispecific construct is suitable for treatment of ovarian cancer or NSCLC.

Embodiment 328. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 329. The multivalent multispecific construct of Embodiment 328, wherein the multivalent multispecific construct is suitable for treatment of NSCLC.

Embodiment 330. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 331. The multivalent multispecific construct of Embodiment 330, wherein the multivalent multispecific construct is suitable for treatment of GI cancer.

Embodiment 332. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 333. The multivalent multispecific construct of Embodiment 332, wherein the multivalent multispecific construct is suitable for treatment of myeloma.

Embodiment 334. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 335. The multivalent multispecific construct of Embodiment 334, wherein the multivalent multispecific construct is suitable for treatment of NHL or autoimmunity.

Embodiment 336. The multivalent multispecific construct of any one of Embodiments 306-325, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 337. The multivalent multispecific construct of Embodiment 336, wherein the multivalent multispecific construct is suitable for treatment of breast cancer.

Embodiment 338. The multivalent multispecific construct of any one of Embodiments 306-337, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

Embodiment 339. The multivalent multispecific construct of any one of Embodiments 306-338, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

Embodiment 340. The multivalent multispecific construct of any one of Embodiments 306-339, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

Embodiment 341. The multivalent multispecific construct of Embodiment 340, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 342. The multivalent multispecific construct of any one of Embodiments 306-341, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6.

Embodiment 343. The multivalent multispecific construct of any one of Embodiments 306-342, wherein L1, L2, L3, and/or L4 is hydrophilic, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.4 to about 0.8, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.5 to about 0.7, optionally wherein L1, L2, L3, and/or L4 has a hydrophobicity of about 0.6 to about 0.65.

Embodiment 344. The multivalent multispecific construct of any one of Embodiments 306-343, wherein L1, L2, L3, and/or L4 is substantially nonimmunogenic.

Embodiment 345. The multivalent multispecific construct of any one of Embodiments 306-344, wherein L1, L2, L3, and/or L4 is substantially non-cleavable.

Embodiment 346. The multivalent multispecific construct of any one of Embodiments 306-345, wherein L1, L2, L3, and/or L4 is substantially cleavable.

Embodiment 347. The multivalent multispecific construct of any one of Embodiments 306-346, wherein L1, L2, L3, and/or L4 is about 5-10 nm long; optionally about 7 nm long.

Embodiment 348. The multivalent multispecific construct of any one of Embodiments 306-347, wherein L3 is a rigid linker, is about 7-12 nm long, and/or comprises an alpha helix.

Embodiment 349. The multivalent multispecific construct of any one of Embodiments 306-348, wherein L2 is a moderately rigid linker, is about 7-12 nm long, and/or comprises b2-microglobulin, Zn-a2-glycoprotein,

tetratricopeptide repeats, or non-alpha helix medium bend and rotational rigidity, optionally wherein L2 is a cleavable linker.

Embodiment 350. The multivalent multispecific construct of any one of Embodiments 306-349, wherein L1 is a flexible linker, is about 7-12 nm long, and/or comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG $C_H2\text{-}C_H3$, or Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15).

Embodiment 351. The multivalent multispecific construct of any one of Embodiments 306-350, wherein the linkers, in order from most to least rigid, are L4, L3, L2, and L1.

Embodiment 352. The multivalent multispecific construct of any one of Embodiments 306-351, wherein the scaffold region comprises a rigid protein.

Embodiment 353. The multivalent multispecific construct of any one of Embodiments 306-352, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG $C_H2\text{-}C_H3$, human serum albumin, human transferrin, or a fragment thereof.

Embodiment 354. The multivalent multispecific construct of any one of Embodiments 306-353, wherein the scaffold region comprises IgG $C_H2\text{-}C_H3$, or a fragment thereof, optionally wherein the IgG $C_H2\text{-}C_H3$ further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-$C_H2\text{-}C_H3$-hinge.

Embodiment 355. The multivalent multispecific construct of any one of Embodiments 306-354, wherein the payload is conjugated to a Cys, a Tyr, or a Lys residue of the scaffold region.

Embodiment 356. The multivalent multispecific construct of any one of Embodiments 306-355, wherein the payload is conjugated to the multivalent multispecific construct using TCEP reduction, transglutaminase, stochastic conjugation, glycan conjugation, lysine conjugation, formylglycine-generating enzyme (FGE) oxidation, AjiCap technology, or click chemistry.

Embodiment 357. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload comprises a protein, optionally wherein the protein comprises an enzyme, an immune stimulant, an immune suppressor, or a topoisomerase.

Embodiment 358. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload comprises a small molecule, optionally wherein the small molecules comprises a small molecule inhibitor, monomethyl auristatin E (MMAE), monomethylauristatin F (MMAF), amanitin, PNU, belotecan, exatecan, pyrrolobenzodiazepine (PBD), ado-trastuzumab emtansine (T-DM1)), a steroid, a chemotherapy, a BCL-XL inhibitor, or a STING agonist.

Embodiment 359. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload comprises an RNA molecule, optionally wherein the RNA molecule is an siRNA molecule.

Embodiment 360. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload comprises a chemosensitizer.

Embodiment 361. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload comprises a genetic modifier.

Embodiment 362. The multivalent multispecific construct of any one of Embodiments 306-356, wherein the payload is a belotecan derivative attached to the scaffold region with a cathepsin cleavable linker.

Embodiment 363. The multivalent multispecific construct of any one of Embodiments 306-362, wherein the multivalent multispecific construct comprises more than one payload, optionally wherein the drug to antibody ratio (DAR) is about 2-8.

Embodiment 364. The multivalent multispecific construct of any one of Embodiments 306-363, wherein the multivalent multispecific construct further comprises one or more enhancer domains and/or one or more FcRn binding domain, optionally wherein the one or more FcRn binding domains comprise IgG Fc C_H2-C_H3 domains or VHH domains.

Embodiment 365. The multivalent multispecific construct of any one of Embodiments 306-364, wherein the diameter of the multivalent multispecific construct is about 5-50 nm, about 10-40 nm, about 20-30 nm, about 20-25 nm, or about 25-30 nm.

Embodiment 366. The multivalent multispecific construct of any one of Embodiments 306-365, wherein each antigen-binding polypeptide contains termini suitable for covalent or non-covalent cyclization or subunit assembly within a producing cell.

Embodiment 367. The multivalent multispecific construct of any one of Embodiments 306-366, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a

higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

Embodiment 368. A nucleic acid encoding the multivalent multispecific construct of any one of Embodiments 306-367.

Embodiment 369. A host cell comprising the nucleic acid of Embodiment 368.

Embodiment 370. A pharmaceutical composition comprising the multivalent multispecific construct of any one of Embodiments 306-367, the nucleic acid of Embodiment 368, or the host cell of Embodiment 369.

Embodiment 371. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-367, the nucleic acid of Embodiment 368, the host cell of Embodiment 369, or the pharmaceutical composition of Embodiment 370.

Embodiment 372. The method of Embodiment 371, wherein the cancer is a solid tumor.

Embodiment 373. The method of Embodiment 372, wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

Embodiment 374. The method of Embodiment 371, wherein the cancer is a hematological cancer.

Embodiment 375. The method of Embodiment 374, wherein the cancer is leukemia, lymphoma, or multiple myeloma.

Embodiment 376. A method of treating ovarian cancer or NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds NaPi2b, ABD B binds MUC1, and ABD A binds mesothelin.

Embodiment 377. A method of treating NSCLC in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds B7H3, ABD B binds PDL1, and ABD A binds Trop2.

Embodiment 378. A method of treating GI cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds Ly6G6D, ABD B binds CEACAM5, and ABD A binds Claudin18.2.

Embodiment 379. A method of treating myeloma in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds BCMA, ABD B binds CD38, and ABD A binds FcRH5.

Embodiment 380. A method of treating NHL or autoimmunity in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds CD22, ABD B binds CD19, and ABD A binds CD79b.

Embodiment 381. A method of treating breast cancer in a subject in need thereof comprising administering the multivalent multispecific construct of any one of Embodiments 306-370, wherein ABD C binds Her2, ABD B binds MUC1, and ABD A binds Trop2.

Embodiment 382. A method of making the multivalent multispecific construct of any one of Embodiments 1-66, 81-141, 156-216, 231-291, and 306-367, comprising culturing a cell expressing the multivalent multispecific construct; and (b) isolating the multivalent multispecific construct from the cultured cell.

EQUIVALENTS

While the invention has been disclosed in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific embodiments disclosed specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

CLAIMS

What is claimed is:

1. A multivalent multispecific construct comprising two or more antigen-binding polypeptides and a conjugated therapeutic payload, wherein each of the two or more antigen-binding polypeptides comprises three or more antigen-binding domains, wherein each of the three or more antigen-binding domains of each of the two or more antigen-binding polypeptides can bind its respective target on a target cell, wherein the construct can bind to four or more targets on the surface of the target cell contemporaneously.

2. The multivalent multispecific construct of claim 1, wherein the construct comprises:
   (a) a therapeutic payload; and
   (b) two or more antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen binding domain (ABD A), optionally a third linker (L3), and a scaffold region;
   wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and
   wherein the scaffold region comprises a ring, a central protein anchor, or an IgG Fc, or a portion of or a modified form thereof.

3. The multivalent multispecific construct of claim 2, wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, wherein the construct comprises two of the antigen-binding polypeptides, wherein each of the two antigen-binding polypeptides further comprises, arranged from N- to C-terminus, (i) ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C, or (ii) L3 – ABD A – L2 – ABD B – L1 – ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C, wherein the N- and C-terminal ABD C domains are the same or different, wherein the N- and C-terminal ABD B domains are the same or different, and wherein the N- and C-terminal ABD A domains are the same or different.

4. The multivalent multispecific construct of claim 2, wherein the scaffold region comprises a rigid polypeptide; wherein the construct comprises 'n' of the antigen-binding polypeptides; wherein each of the antigen-binding polypeptides further comprises a fourth linker (L4), wherein L4 attaches the scaffold region to ABD C; and wherein the scaffold region further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring that forms a closed ring, wherein n is 3-12.

5. A multivalent multispecific construct comprising:
   (a) a therapeutic payload; and
   (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, optionally a second L3, a second ABD A, a second L2, a second ABD B, a second L1, and a second ABD C;

   wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – ABD A – L2 – ABD B – L1 – ABD C or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L3 – ABD A – L2 – ABD B – L1 – ABD C; and

   wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof, wherein the N- and C-terminal ABD C domains are the same or different, wherein the N- and C-terminal ABD B domains are the same or different, and wherein the N- and C-terminal ABD A domains are the same or different.

6. A multivalent multispecific construct comprising:
   (a) a therapeutic payload; and
   (b) two antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region;

   wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and

wherein the scaffold region comprises an IgG Fc, or a portion of or a modified form thereof.

7. A multivalent multispecific construct comprising:
   (a) a therapeutic payload; and
   (b) four antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), and a scaffold region;

   wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, and L3 optionally attaches ABD A to the scaffold region, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region; and

   wherein the scaffold region comprises a central protein anchor, optionally wherein the central protein anchor comprises an FcRn binding domain.

8. A multivalent multispecific construct comprising:
   (a) a therapeutic payload; and
   (b) 'n' antigen-binding polypeptides that target two or more different targets, each comprising, from N- to C-terminus, a first antigen-binding domain (ABD C), a first linker (L1), a second antigen-binding domain (ABD B), a second linker (L2), a third antigen-binding domain (ABD A), optionally a third linker (L3), a scaffold region, and a fourth linker (L4);

   wherein L1 attaches ABD C to ABD B, L2 attaches ABD B to ABD A, L3 optionally attaches ABD A to the scaffold region, and L4 attaches the scaffold region to ABD C, such that each antigen-binding polypeptide comprises, arranged from N- to C-terminus, (i) ABD C – L1 – ABD B – L2 – ABD A – scaffold region – L4 or (ii) ABD C – L1 – ABD B – L2 – ABD A – L3 – scaffold region – L4;

   wherein the scaffold region comprises a rigid polypeptide;

   wherein the construct further comprises a clasp domain that attaches the first and nth antigen-binding polypeptides to form a closed ring; and

   wherein n is 3-12.

9. The multivalent multispecific construct of any one of claims 2-8, wherein ABD C, ABD B, and/or ABD A is a VHH, a VNAR, a Fab, a ScFv, a nanobody, a diabody, a synthetic minibody, a ligand, or a binding fragment thereof.

10. The multivalent multispecific construct of any one of claims 2-9, wherein ABD C, ABD B, and/or ABD A is a conditional binder, optionally wherein binding of ABD C, ABD B, and/or ABD A to their respective targets depends on pH, calcium, or ATP levels.

11. The multivalent multispecific construct of any one of claims 2-10, wherein ABD C, ABD B, and/or ABD A targets a specific cell type, optionally wherein ABD C, ABD B, and/or ABD A targets cancer cells or immune cells.

12. The multivalent multispecific construct of any one of claims 2-11, wherein ABD C, ABD B, and/or ABD A promotes internalization of the multivalent multispecific construct by the target cell, optionally by promoting lysosomal trafficking of the multivalent multispecific construct by the target cell, optionally wherein the lysosomal trafficking is clathrin-mediated endocytosis or receptor-mediated endocytosis.

13. The multivalent multispecific construct of any one of claims 2-12, wherein ABD C, ABD B, and/or ABD A promotes chemotherapy sensitization of the target cell, and/or wherein ABD C, ABD B, and/or ABD A promotes apoptosis or necrosis of the target cell.

14. The multivalent multispecific construct of any one of claims 2-13, wherein ABD C, ABD B, and/or ABD A comprises at least one half-life extending molecule, optionally wherein the half-life extending molecule is an FcRn binding domain, albumin, PEG, XTEN, or a human serum albumin (HSA) binding domain.

15. The multivalent multispecific construct of any one of claims 2-13, wherein the scaffold region comprises IgG C_H2-C_H3, or a fragment thereof, optionally wherein the IgG C_H2-C_H3 further comprises a second hinge region, optionally wherein the scaffold region comprises hinge-C_H2-C_H3-hinge.

16. The multivalent multispecific construct of any one of claims 2-15, wherein ABD C, ABD B, ABD A, L1, L2, L3, L4, hinge region, second hinge region, and/or the scaffold region comprises at least one site for conjugation of the payload, optionally wherein the at least one site comprises a Cys, a Tyr, or a Lys residue or an engineered glycan or tag for site specific conjugation.

17. The multivalent multispecific construct of any one of claims 2-16, wherein ABD C binds to a first target molecule, ABD B binds to a second target molecule, and/or ABD A binds to a third target molecule, optionally wherein the N-terminal ABD C binds to a first target molecule, the N-terminal ABD B binds to a second target molecule, the N-terminal ABD A binds to a third target molecule, the C-terminal ABD C binds to a fourth target molecule, the C-terminal ABD B binds to a fifth target molecule, and/or the C-terminal ABD A binds to a sixth target molecule.

18. The multivalent multispecific construct of any one of claims 2-17, wherein ABD C binds to a first epitope on a first target molecule, ABD B binds to a second epitope on the first target molecule, and/or ABD A binds to a third epitope on the first target molecule.

19. The multivalent multispecific construct of any one of claims 2-18, wherein L1, L2, L3, and/or L4 is about 15-30 aa long.

20. The multivalent multispecific construct of any one of claims 2-19, wherein L1, L2, L3, and/or L4 comprises one or more of Gly, Ser, Thr, Pro, Arg, Phe, Glu, Cys, Asn, Ala, Asp, and Gln residues, optionally wherein L1, L2, L3, and/or L4 primarily comprises Gly, Ser, and Thr residues.

21. The multivalent multispecific construct of any one of claims 2-20, wherein L1, L2, L3, and/or L4 primarily comprises Gly and Ser residues.

22. The multivalent multispecific construct of claim 21, wherein L1, L2, L3, and/or L4 comprises $(Gly\text{-}Ser)_n$, $(Gly_4\text{-}Ser)_n$ (SEQ ID NO: 1), $(Gly_3\text{-}Ser)_n$ (SEQ ID NO: 2), Gly-Gly-Ser, Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Gly-Gly (SEQ ID NO: 4), Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 5), Gly-Gly-Gly-Gly-Gly-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly-Gly-Gly-Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 7), Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 8), Gly-Ser-Gly-Ser-Gly-Ser (SEQ ID NO: 9), $(Gly_4\text{-}Ser)_3$, (SEQ ID NO: 10), $(Gly_2\text{-}Ser)_3$ (SEQ ID NO: 11), $(Gly_2\text{-}Ser)_2$ (SEQ ID NO: 12), Ferritin E Helix, $(Glu\text{-}Ala\text{-}Ala\text{-}Ala\text{-}Lys)_{1\text{-}3}$ (SEQ ID NO: 13), $(Ala\text{-}Pro)_n$ (SEQ ID NO: 14), IgG C_H1, IgG C_H2-C_H3, Ser-Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 15), or Gly-Gly-Ser-Asn-Gly-Ser-Gly-Gly-Ser (SEQ ID NO: 28).

23. The multivalent multispecific construct of any one of claims 2-22, wherein the scaffold region comprises a rigid protein.

24. The multivalent multispecific construct of any one of claims 2-23, wherein the scaffold region comprises a moiety for extending PK, optionally wherein the moiety for extending PK is an FcRn binding domain, an IgG C_H2-C_H3, human serum albumin, human transferrin, or a fragment thereof.

25. The multivalent multispecific construct of any one of claims 2-24, wherein the multivalent multispecific construct (i) delivers payloads to target cells more efficiently than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, (ii) is internalized by target cells at a higher rate than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A, and/or (iii) has improved specificity for binding to and/or killing tumor cells than a monomeric or a monospecific binder having only one copy of ABD C, ABD B, or ABD A.

26. A nucleic acid encoding the multivalent multispecific construct of any one of claims 1-25.

27. A host cell comprising the nucleic acid of claim 26.

28. A pharmaceutical composition comprising the multivalent multispecific construct of any one of claims 1-25, the nucleic acid of claim 26, or the host cell of claim 27.

29. A method of treating a disease or disorder, optionally wherein the disease or disorder is a cancer, in a subject in need thereof comprising administering the multivalent multispecific construct of any one of claims 1-25, the nucleic acid of claim 26, the host cell of claim 27, or the pharmaceutical composition of claim 28.

30. The method of claim 29, wherein the cancer is a solid tumor, optionally wherein the cancer is NSCLC, ovarian cancer, breast cancer, pancreatic cancer, colorectal cancer, or gastric cancer.

31. The method of claim 29, wherein the cancer is a hematological cancer, optionally wherein the cancer is leukemia, lymphoma, or multiple myeloma.