Bispecific/Biparatopic Antibodies in Breast Cancer

MaTOS
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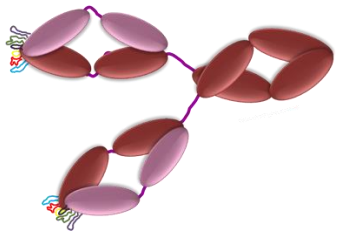
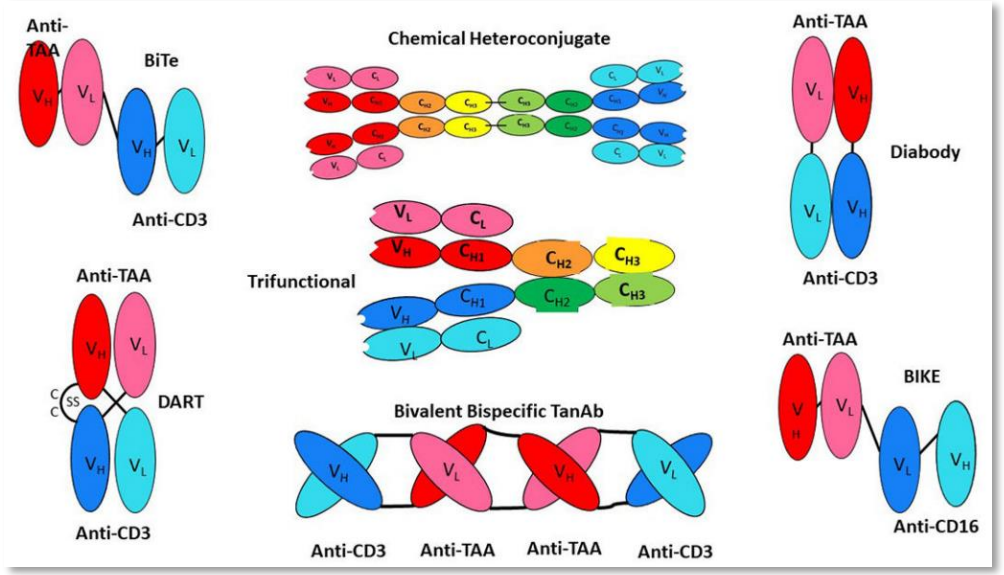
What are bispecific antibodies (BsAbs)?

- BsAbs are engineered immune constructs with two distinct binding domains that can bind to two antigens or two epitopes of the same antigen simultaneously.[1]
- There are over 100 BsAbs in clinical development.[2]
- In 2021, FDA finalized a guidance on BsAb development programs, which discusses aspects for chemistry, manufacturing, and controls (CMCs), as well as immunogenicity. [3]
- Biparatopic antibody represents a subset of BsAbs that bind distinct, non-overlapping epitopes on the same target antigen.[13]

IgG Schema[31]

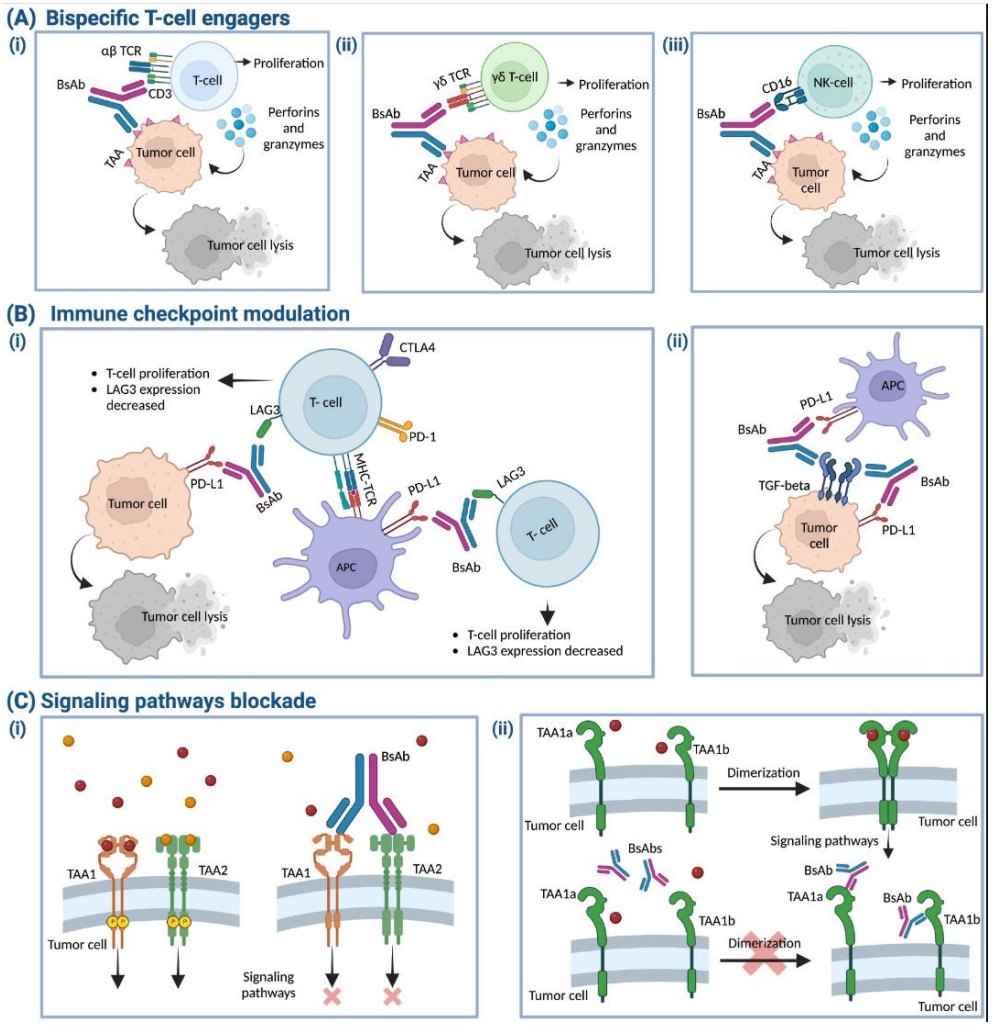


Schema with various BsAbs formats [8]



1) US Food and Drug Administration. Bispecific antibodies: An Area of Research and Clinical Applications 2024. Accessed Feb 3, 2025.
2) Brinkmann U, Kontermann RE. Bispecific antibodies. Science. 2021 May. 916-917
3) US Food and Drug Administration. FDA guidance: Bispecific antibody development programs. 2021 May. Accessed Feb 3, 2025.
7) Heater NK, Warrior S, Lu J. Current and future immunotherapy for breast cancer. Journal of Hematology & Oncology 2024.
8) Dillon PM, Tushir-Singh J, Lum LG. Bispecific Antibodies For the Treatment of Breast Cancer. Expert Opin Biol Ther. 2022
13) Niquille DL, Fitzgerald KM, Gera N. Biparatopic antibodies: therapeutic applications and prospects. MAbs.
31) Pohlmann et al. CCR Focus 2009

What is the mechanism of action of BsAbs? [9]



(A) Bispecific T cell engagers.

- BsAb binds the CD3 subunit of the TCR and the selected TAA to facilitate the formation of a synapse between the two cell types; the synapse activates T cells, which can release perforins and granzymes to lyse tumor cells.
- Bispecific γδ T cell engagers simultaneously bind Vγ9Vδ2 receptors and a specific TAA to activate T cells, release perforins and granzymes, and lyse tumor cells.
- Bispecific NK cell engagers simultaneously bind CD16 receptors and a specific TAA to activate NK cells, release perforins and granzymes, and lyse tumor cells.

(B) Immune checkpoint (ICP) modulation.

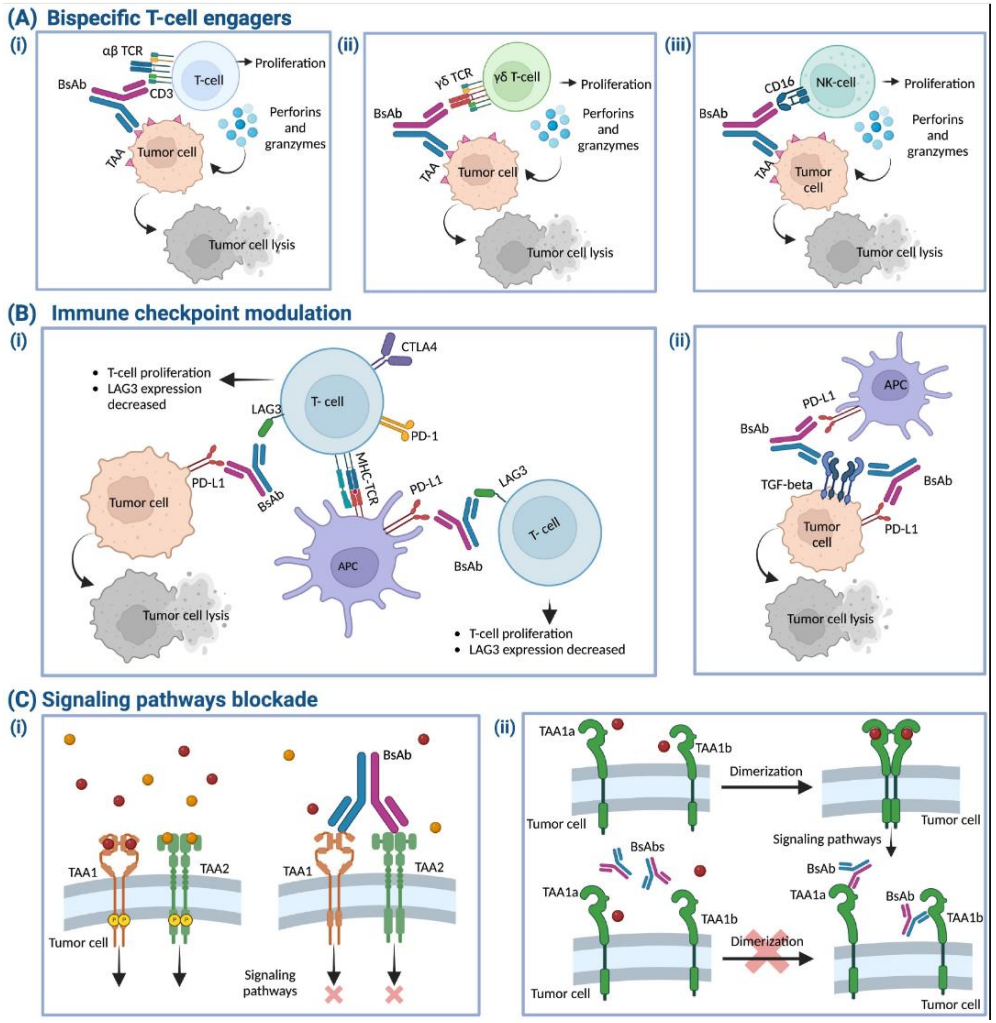
- Dual ICP-blocking bsAbs simultaneously bind lymphocyte-activation gene 3 (LAG3) on the surface of T cells and programmed death ligand 1 (PD-L1) on tumor or APC, amplifying the activation of T cells, which release perforins and granzymes, and lyse tumor cell, decreasing LAG3 expression.
- BsAb targets ICP concurrently with a molecule involved in other signaling pathways.

(C) Signaling pathway blockade.

- BsAbs simultaneously bind EGFR and cMET, blocking the ligand-induced phosphorylation, promoting inhibition of the downstream signaling cascades, and stimulating receptor degradation.
- Biparatopic bsAbs bind two separate epitopes on the same target.

9) Herrera M, Pretelli G, Desai J, Garralda E, Siu LL, Steiner TM, Au L. Bispecific antibodies: advancing precision oncology. Trends in Cancer 2024.

What are the mechanisms of resistance of BsAbs? [9]



Immune checkpoint proteins and costimulatory molecules

- Expression of the inhibitory ligands PD-L1 and PD-L2 reduces the cytolytic activity of BsAbs [10,11,12]
- These data have prompted early-phase clinical trials of TCEs combined with anti-PD-1 antibodies in Hematology [9]

Loss of target antigen expression

- Loss of CD19 at PD after blinatumomab (disruption of CD19 membrane trafficking in the post-endoplasmic reticulum)
- Loss of CD20 at relapse following glofitamab treatment (in aggressive B-NHL).

MHC-I downregulation or loss and impaired IFN-γ signaling

- IFN-γ increases the sensitivity of redirected T cell cancer cell killing with bsAb treatment

Anti-drug antibodies (ADAs)

- Drug resistance from **anti-drug antibodies (ADAs)** results from formation of neutralizing antibodies (NAbs) against the variable regions of the bsAb, preventing target antigen engagement.

9) Herrera M, Pretelli G, Desai J, Garralda E, Siu LL, Steiner TM, Au L. Trends in Cancer 2024.
10) Topp MS et al. Blood. 2017
11) Laszlo GS et al. Blood Cancer J. 2015
12) Krupka C et al. Leukemia. 2016

Bispecific antibodies approved in oncology

BsAb	Target	Indication	Approval year
Blinatumomab	CD3 × CD19	ALL Ph^+; MRD+ ALL	2014
Amivantamab	EGFR × MET	NSCLC with EGFR exon 20 insertion mutations	2021
Teclistamab	BCMA × CD3	Relapsed/Refractory Multiple Myeloma	2022
Mosunetuzumab	CD20 × CD3	Non-Hodgkin Lymphoma	2022
Tebentafusp	gp100 × CD3	Uveal Melanoma (HLA-A*02:01-positive)	2022
Elranatamab	BCMA × CD3	Relapsed/Refractory Multiple Myeloma	2023
Talquetamab	GPRC5D × CD3	Relapsed/Refractory Multiple Myeloma	2023
Glofitamab	CD20 × CD3	B-cell Malignancies	2023
Epcoritamab	CD20 × CD3	Diffuse Large B-cell Lymphoma (DLBCL)	2023
Tarlatamab	CD3 × DDL3	ES-SCLC after platinum-based chemotherapy	2024
Odronextamab	CD20/CD3	R/R FL & R/R DLBCL	2024 (EMA)
Zanidatamab	HER2 (Two Epitopes)	HER2-positive Biliary Tract Cancer (BTC)	2024
Zenocutuzumab	HER2 x HER3	advanced, unresectable or metastatic pancreatic adenoca or NSCLC harboring an NRG1 gene fusion	2024

Bispecific antibodies in Breast Oncology

BsAbs

Zanidatamab (ZW25)
KN026
PM8002
Zenocutuzumab

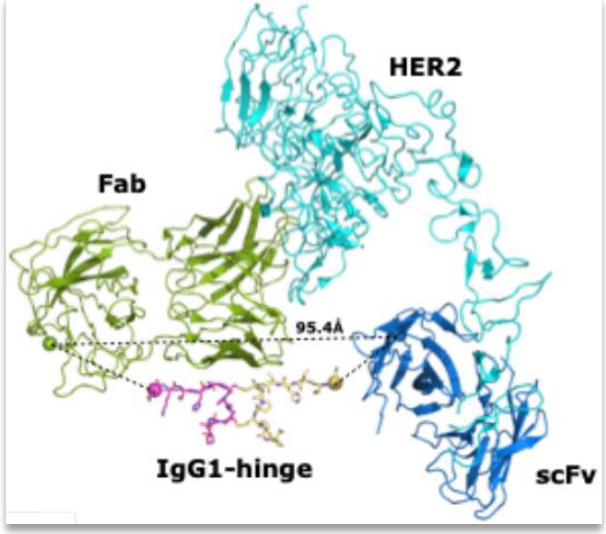
BsADCs

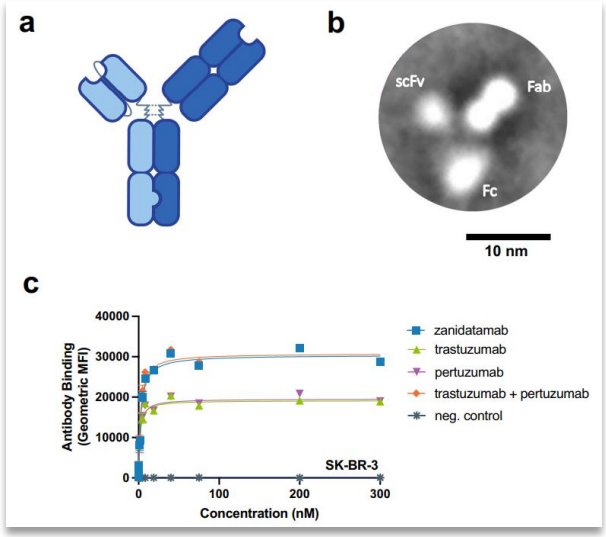
Zanidatamab Zovodotin (ZW49)
BL-B01D1

Zanidatamab (ZW25): mechanisms of action [19]

- ZW25 is a humanized anti-HER2 IgG1 bispecific biparatopic antibody that simultaneously binds two HER2 epitopes, ECD4 and ECD2 (**a** & **b**).

- ZW25 binds HER2-expressing tumor cells with greater Ab saturation than trastuzumab or pertuzumab (**c**).[19]



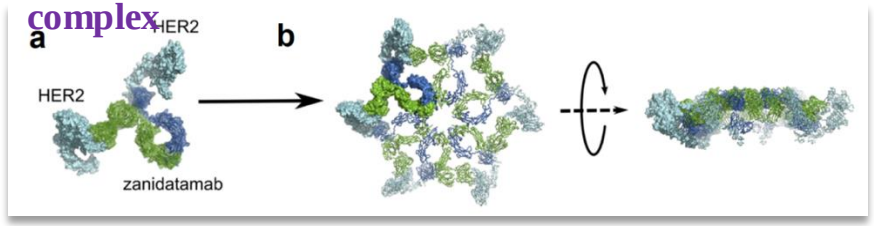


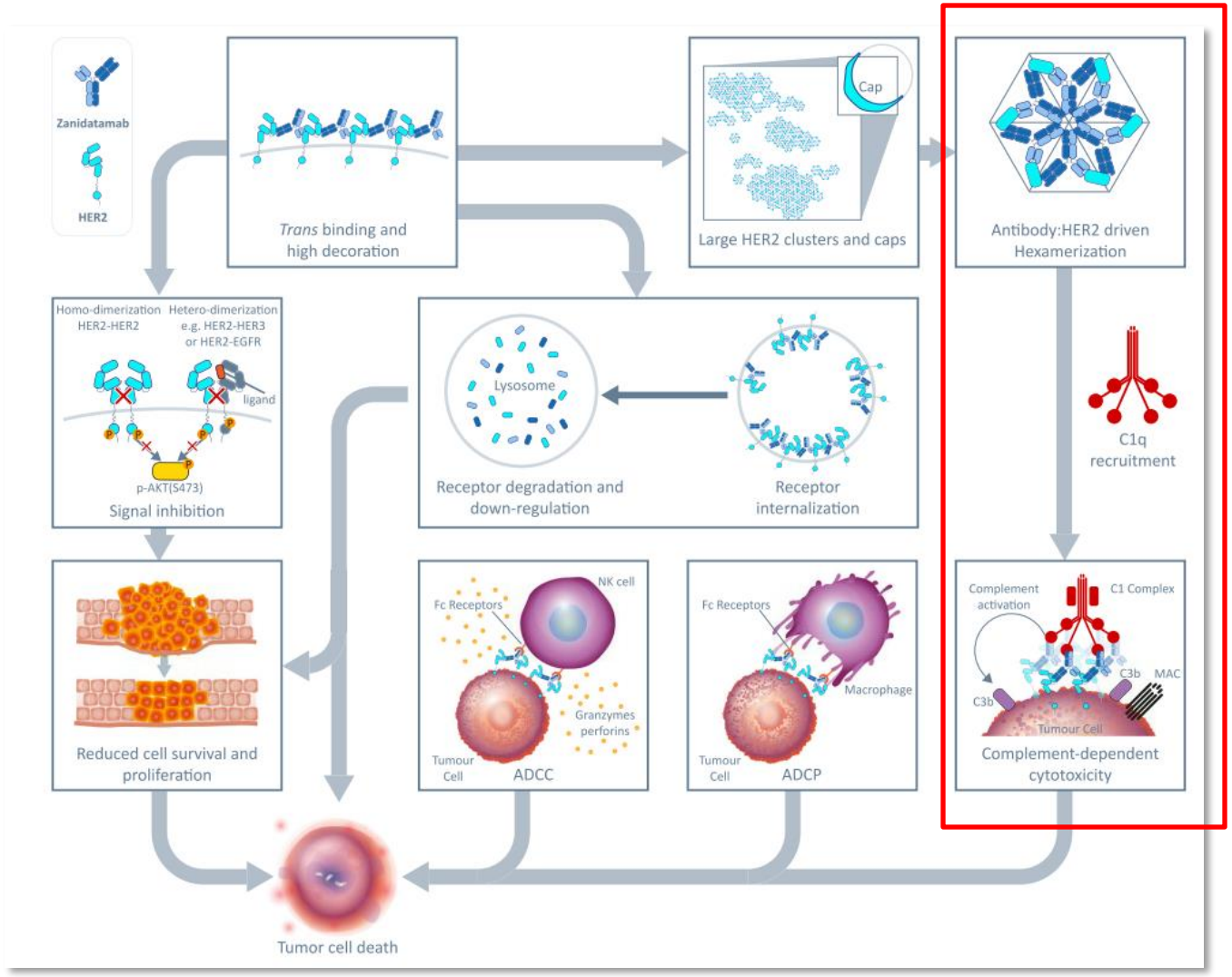
19) Weisser NE et al. An anti-HER2 biparatopic antibody that induces unique HER2 clustering and complement-dependent cytotoxicity. Nat Commun 2023.

Zanidatamab (ZW25): mechanisms of action [19]

- It mediates HER2 internalization and degradation, inhibition of both cell signaling and tumor growth.
- It elicits potent **CDC**, ADCC and ADCP against high HER2-expressing tumor cells in vitro.
  - ✓ Engagement of the classical complement pathway is governed by several factors, including antigen size and density, that impact the ability of the antigen-Ab complex to assume a geometry that allows efficient C1q binding [20]
  - ✓ Optimal CDC activity requires hexameric organization of Ab Fc domains in the Ab-antigen clusters [21]

Hexamerization model of zanidatamab:HER2 complex



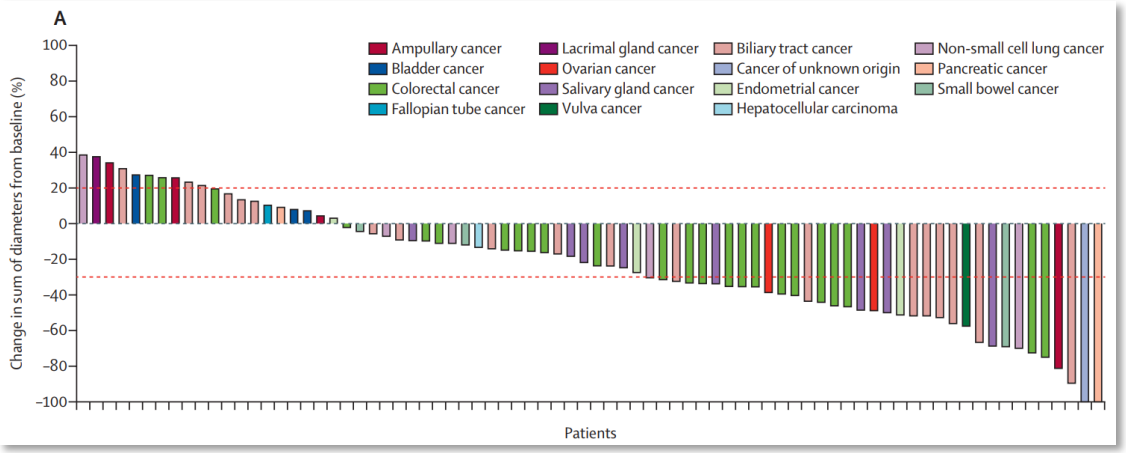


19) Weisser NE et al. Nat Commun 2023.
20) Merle NS et al. Front. Immunol. 6, 262 (2015).
21) Diebolder CA et al. Science 343, 1260–1263 (2014)

Zanidatamab: efficacy

Metastatic setting

In a phase I trial, monotherapy with ZW25 in heavily pretreated patients with HER2+ solid tumors led to ORR: breast cancer, 33%; GE cancer, 44%; other tumor types, 33%; including CRC and gallbladder cancer. [14,22]



Neoadjuvant setting

- Single institution Phase 2 pilot trial (NCT05035836) [18]
- Stage I HER2+ breast cancer
- Single agent zanidatamab x3 neoadjuvant cycles
- Chemotherapy free regimen
- Efficacy:
  - pCR = 36%
  - RCB 0-1 rate = 64%
- Safety:
  - Well-tolerated
  - No grade 3-5 toxicity

14) Meric-Bernstam F et al Single agent activity of ZW25, a HER2-targeted bispecific antibody, in heavily pretreated HER2-expressing cancers. J Clin Oncol 36:15s; 2018 (suppl; abstr 2500).
22) Meric-Bernstam F et al. Zanidatamab, a novel bispecific antibody, for the treatment of locally advanced or metastatic HER2-expressing or HER2-amplified cancers: a phase 1, dose-escalation and expansion study. Lancet Oncol 2022.
18) Valero V, Mouabbi J, Alonzo H, et al. Neoadjuvant zanidatamab for stage I node negative HER2 positive breast cancer (BC). Presented at: 2023 ESMO Breast Cancer Annual Congress; May 11-13, 2023; Berlin, Germany. Abstract 132P.

Zanidatamab: efficacy

- In a phase 1b/2 study, Zanidatamab + *Docetaxel* ORR = 90.9% in patients with advanced HER2+ mBC.[16]
- In HR+/HER2+ patients with advanced disease, the combination of zanidatamab with *palbociclib and fulvestrant* achieved a median PFS of 11.3 months with an ORR of 34.5%. [17]
- An upcoming phase III trial (NCT06435429) will investigate *chemotherapy* with zanidatamab or trastuzumab for patients with HER2+ disease who have progressed on T-DXd.

14) Meric-Bernstam F, Beeram M, Mayordomo JI, Hanna DL, Ajani JA, Murphy MAB, et al Single agent activity of ZW25, a HER2-targeted bispecific antibody, in heavily pretreated HER2-expressing cancers. J Clin Oncol 36:15s; 2018 (suppl; abstr 2500).
16) Wang X, Lee KS, Zeng X, Sun T, Im YH, Li H, Wang K, Li H, et al. Zanidatamab (zani), a HER2-targeted bispecific antibody, in combination with docetaxel as first-line therapy (1L) for patients (pts) with advanced HER2-positive breast cancer (BC): Updated results from a phase 1b/2 study. J Clin Oncol. 2023;41(16_suppl):1044-1044.
17) Escrivá-de-Romani S, Alba E, Rodríguez-Lescure Á, Hurvitz S, Cejalvo JM, Gión M, et al. Abstract PD18-10: Treatment of HER2-positive (HER2+) hormone-receptor positive (HR+) metastatic breast cancer (mBC) with the novel combination of zanidatamab, palbociclib, and fulvestrant. Cancer Res. 2023;83(5_Supplement):PD18-10-PD18-10.

Zanidatamab: safety

Monotherapy treatment related adverse events [22]

	Part 1: dose escalation (n=46)		Part 2: dose expansion (n=86)	
	Grade 1–2	Grade 3	Grade 1–2	Grade 3
Diarrhea	24 (52%)	0	36 (42%)	1 (1%)
Infusion reaction	20 (43%)	0	29 (34%)	0
Nausea	9 (20%)	0	8 (9%)	0
Fatigue	8 (17%)	1 (2%)	8 (9%)	0
Vomiting	5 (11%)	0	6 (7%)	0
Decreased appetite	2 (4%)	1 (2%)	2 (2%)	0
Arthralgia	1 (2%)	1 (2%)	0	0
Hypertension	0	1 (2%)	0	0
Hypophosphatemia	0	1 (2%)	0	0

- No DLTs
- MTD not reached
- RP2D 20mg/Kg IV Q2W
- Grade 3 treatment related adverse events: 6 events in 4/132 patients
- No treatment related deaths

22) Meric-Bernstam F et al. Zanidatamab, a novel bispecific antibody, for the treatment of locally advanced or metastatic HER2-expressing or HER2-amplified cancers: a phase 1, dose-escalation and expansion study. Lancet Oncol 2022.

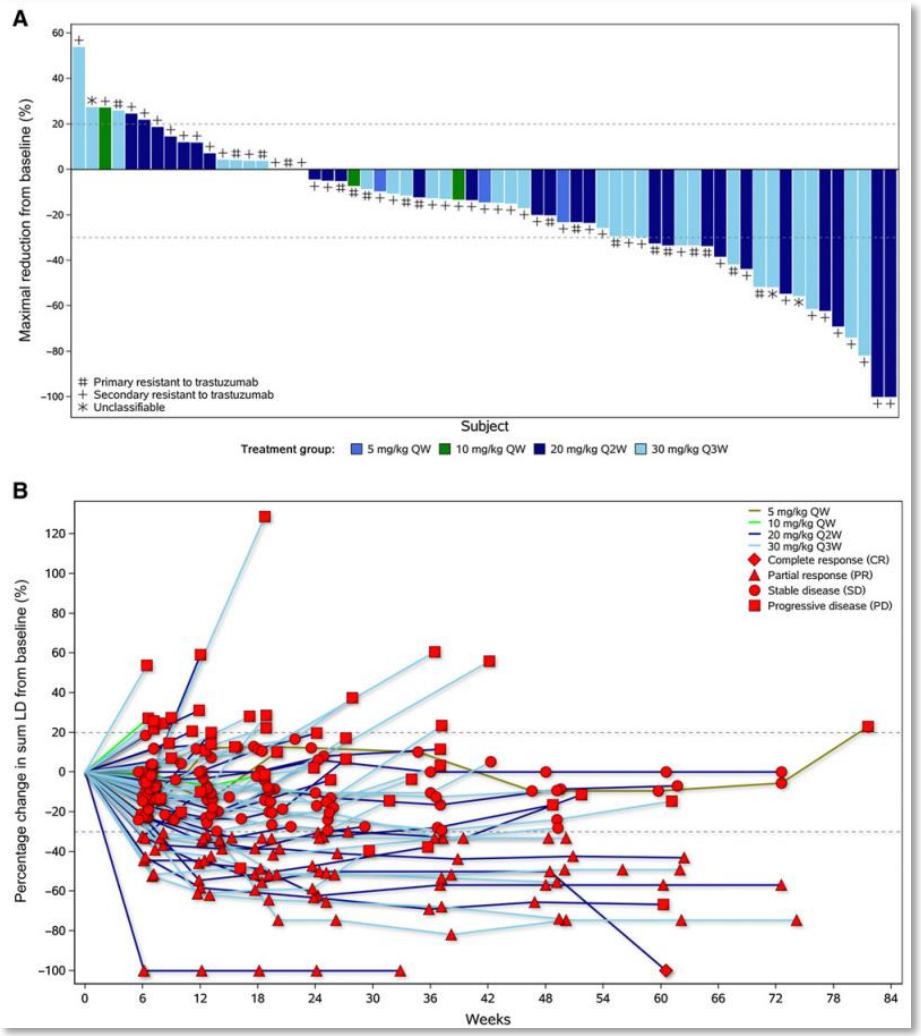
KN026

- Dual-HER2 bispecific antibody generated from heavy chains of trastuzumab and pertuzumab, binding to ECD4 and ECD2 of HER2.
- FIH trial KN026-CHN001 study [NCT03619681]: [29]
  - Patients with HER2+ mBC
  - ORR 25.4%
  - mPFS 5.6 months

Most common TRAEs (any and grade 3/4) that occurred in 10% or more patients (safety analysis set).

	5 mg/kg QW (*N* = 3)		10 mg/kg QW (*N* = 3)		20 mg/kg Q2W (*N* = 28)		30 mg/kg Q3W (*N* = 29)		Total (*N* = 63)	
	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3	Total	Grade ≥ 3	Total
Subjects with at least 1 TRAE	0	3 (100%)	0	2 (66.7%)	2 (7.1%)	26 (92.9%)	2 (6.9%)	25 (86.2%)	4 (6.3%)	56 (88.9%)
Pyrexia	0	1 (33.3%)	0	1 (33.3%)	0	8 (28.6%)	0	5 (17.2%)	0	15 (23.8%)
Diarrhea	0	1 (33.3%)	0	1 (33.3%)	0	6 (21.4%)	0	6 (20.7%)	0	14 (22.2%)
Alanine aminotransferase increased	0	0	0	0	0	8 (28.6%)	0	6 (20.7%)	0	14 (22.2%)
Aspartate aminotransferase increased	0	0	0	0	0	6 (21.4%)	0	8 (27.6%)	0	14 (22.2%)
White blood cell count decreased	0	2 (66.7%)	0	0	0	3 (10.7%)	0	6 (20.7%)	0	11 (17.5%)
Hypokalemia	0	2 (66.7%)	0	0	0	2 (7.1%)	0	4 (13.8%)	0	8 (12.7%)
Infusion-related reaction	0	0	0	0	0	3 (10.7%)	1 (3.4%)	5 (17.2%)	1 (1.6%)	8 (12.7%)
Neutrophil count decreased	0	1 (33.3%)	0	0	0	4 (14.3%)	0	3 (10.3%)	0	8 (12.7%)
Rash	0	0	0	0	0	4 (14.3%)	0	3 (10.3%)	0	7 (11.1%)
Transaminases increased	0	0	0	0	1 (3.6%)	1 (3.6%)	0	0	1 (1.6%)	1 (1.6%)
Ventricular arrhythmia	0	0	0	0	1 (3.6%)	1 (3.6%)	0	0	1 (1.6%)	1 (1.6%)
Cardiac myxoma	0	0	0	0	0	0	1 (3.4%)	1 (3.4%)	1 (1.6%)	1 (1.6%)

Abbreviations: QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks; TRAE, treatment-related adverse events.



29. Zhang J, Ji D, Cai L, Yao H, Yan M, Wang X, et al. First-in-human HER2-targeted bispecific antibody KN026 for the treatment of patients with HER2-positive metastatic breast cancer: results from a phase I study. Clin Cancer Res. 2022.

KN026 + chemotherapy; neoadjuvant setting

KN026 30 mg/Kg + docetaxel 75 mg/m^2 IV Q3W x 4 cycles: regimen tested in the neoadjuvant setting [NCT04881929]. [30]

- N=30
- Stage IIIA 47%
- HR+ 50%

Efficacy:

- pCR rate 56.7%
- ORR 90% (95% CI, 73.47%-97.89%), and the confirmed ORR was 86.7% (95% CI, 69.28%-96.24%).

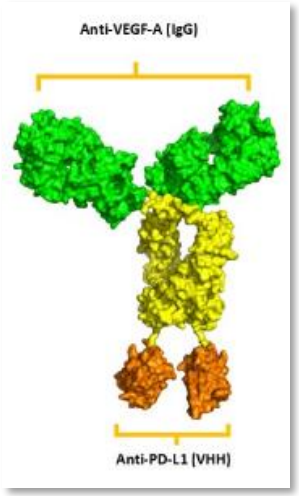
Safety:

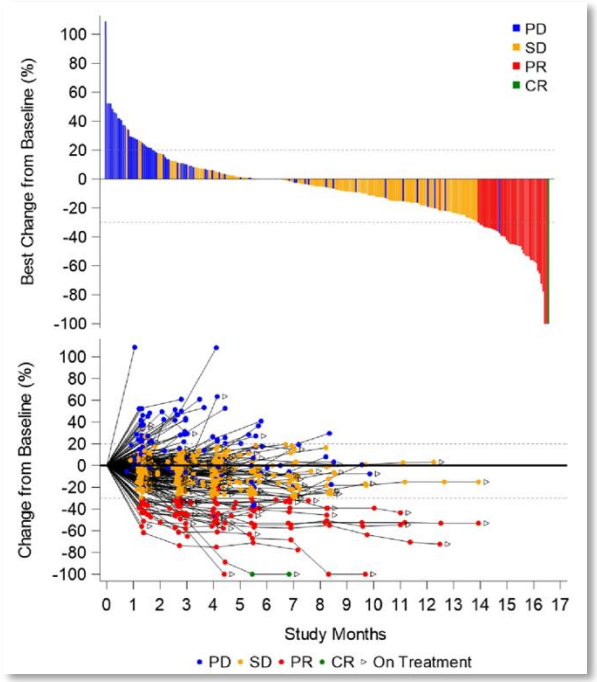
- 2 patients discontinued treatment due to toxicity
- AEs included neutropenia (50.0%), leukopenia (40.0%), lymphopenia (10.0%), increased GGT (3.3%), increased ALT (3.3%), febrile neutropenia (3.3%), hepatitis E (3.3%), dermatitis acneiform (3.3%), diarrhea (3.3%), and hypersensitivity (3.3%)

30. Ma L, Yang B, Zhang M, Wang K, Chen Y, Fan Z, et al. 247P KN026 in combination with docetaxel as neoadjuvant treatment for HER2+ early or locally advanced breast cancer (BC): a single-arm, multicenter, phase II study. Ann Oncol. 2023;34:S282.

PM8002/BNT327 monotherapy

- PM8002 is a bispecific antibody targeting PD-L1 and VEGF-A:
  - ✓ It has 2 humanized VHHs against PD-L1 fused to the c-terminus of an anti-VEGF-A IgG
- PM8002 blocks the interaction of PD-L1/PD-1, reversing immunosuppressive phenotype, while scavenging and neutralizing VEGF-A released by tumor cells
- Dose expansion monotherapy in solid tumors (China) presented at ASCO 2023.[24]
  - ✓ SAE: 11.3% of patients
  - ✓ AESI: hemorrhage (4.8%), thromboembolism (0.3%), GI perforation/fistula (0.6%)
  - ✓ No grade 4-5 AEs
  - ✓ ADA: 1.7% (4 patients)



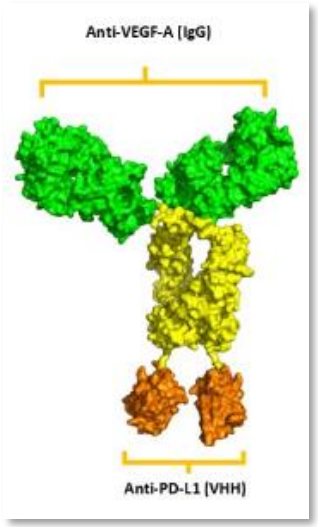


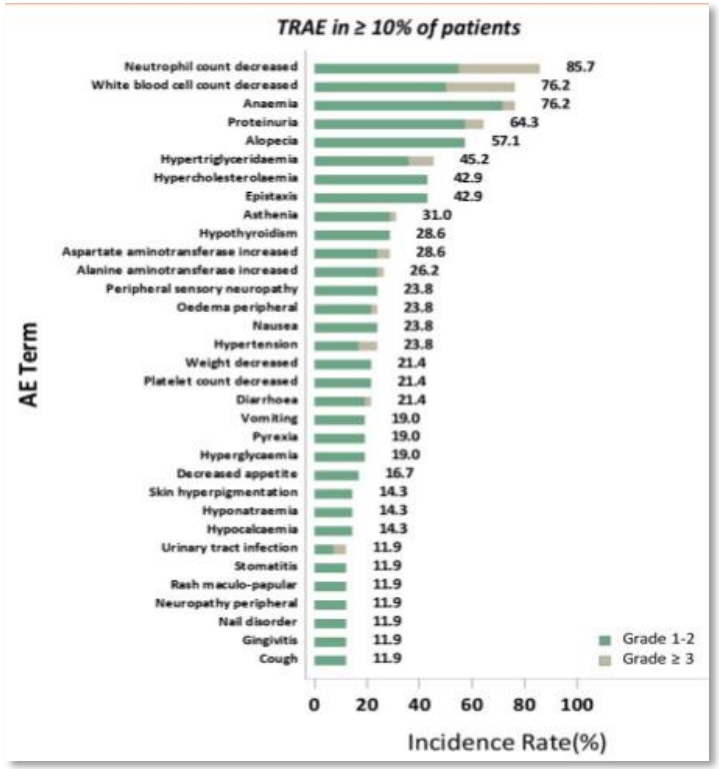
Categories	n (%)			
All TRAE	239 (77.1)			
≥3 TRAE	64 (20.6)			
SAE	35 (11.3)			
TRAE leading to discontinuation	17 (5.5)			
TRAE ≥ 10%	**Grade, n (%)**			
	All Grades	**Grade 3**	**Grade 4**	**Grade 5**
Aspartate aminotransferase increased	42 (13.5)	2 (0.6)	0	0
Alanine aminotransferase increased	39 (12.6)	1 (0.3)	0	0
Hypercholesteremia	38 (12.3)	0	0	0
Hypoalbuminemia	35 (11.3)	0	0	0
Hypertriglyceridemia	31 (10.0)	2 (0.6)	0	0
Proteinuria	82 (26.5)	4 (1.3)	0	0
Hypertension	60 (19.4)	20 (6.5)	0	0
Hypothyroidism	34 (11.0)	1 (0.3)	0	0
Anaemia	32 (10.3)	0	1 (0.3)	0
Related AESI				
Haemorrhage	15 (4.8)	2 (0.6)	0	0
Thromboembolic	1 (0.3)	0	0	0
Gastrointestinal perforations & fistula	2 (0.6)	2 (0.6)	0	0

24) Guo Y et al. ASCO 2023.

PM8002/BNT327 + nab-paclitaxel

- Completed Phase Ib/II trial PM8002-B004C + Nab-paclitaxel for 1L TNBC [NCT05918133][23]
  - ✓ Fudan University, China
  - ✓ TNBC n=42
- Ongoing Phase III trial PM8002-C013C-TNBC-R [NCT06419621]
  - ✓ Nab-paclitaxel + PM8002 or placebo
  - ✓ 70 study locations in China



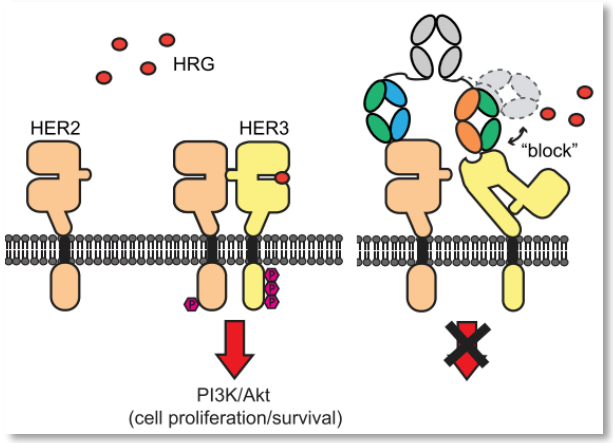


Variable	ITT	PD-L1 CPS<1	PD-L1 1≤CPS<10	PD-L1 CPS≥10	NOT DONE
Population (n)	42	13	16	9	4
CR	1 (2.4)	0	1 (6.3)	0	0
PR	32 (76.2)	10 (76.9)	10 (62.5)	9 (100)	3 (75.0)
SD	7 (16.7)	3 (23.1)	4 (25.0)	0	0
PD	2 (4.8)	0	1 (6.3)	0	1 (25.0)
ORR % (95% CI)	78.6 (63.2, 89.7)	76.9 (46.2, 95.0)	68.8 (41.3, 89.0)	100 (66.4, 100)	75.0 (19.4, 99.4)
cORR % (95% CI)	73.8 (58.0, 86.1)	76.9 (46.2, 95.0)	56.3 (29.9, 80.3)	100 (66.4, 100)	75.0 (19.4, 99.4)
DCR % (95% CI)	95.2 (83.8, 99.4)	100 (75.3, 100)	93.8 (69.8, 99.8)	100 (66.4, 100)	75.0 (19.4, 99.4)
mPFS (Mo), (95%CI)	13.5 (9.4, 19.3)	18.1 (5.7, --)	14.0 (7.2, --)	10.8 (5.5, 13.5)	14.0 (1.8, --)

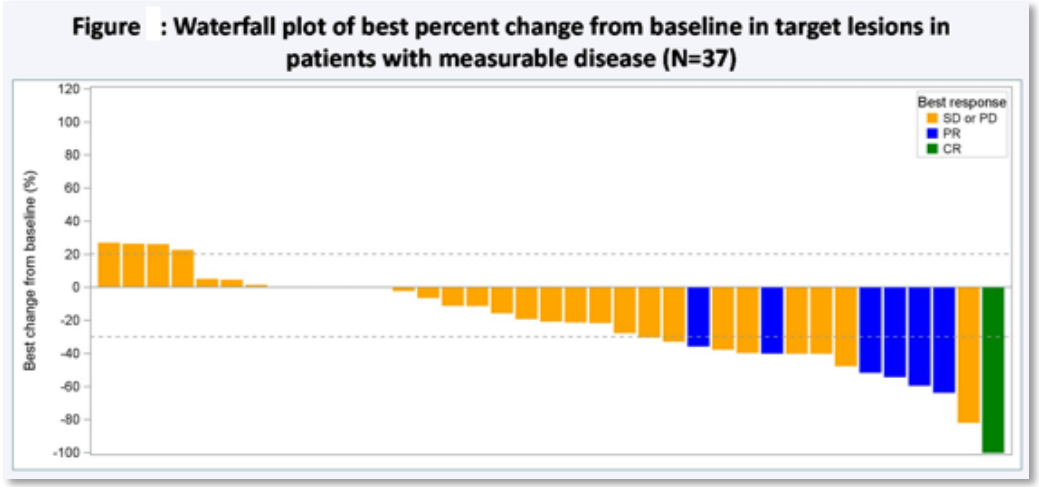
23) Wu J et al. SABCS 2024.

MCLA-128/Zenocutuzumab

- BsAb targeting HER2 and HER3
- Inhibits HER3 ligand interaction and prevents HER2/HER3 heterodimerization and PI3K/AKT/mTOR pathway activation.[25]
- Approved in advanced, unresectable or metastatic pancreatic adenocarcinoma or NSCLC harboring an NRG1 gene fusion.
  - ✓ NRG1 fusion frequency in breast cancer (1-6%)[26]
- In breast:
  - ✓ In a phase II trial of zenocutuzumab + ET in patients with HR+HER2-low MBC previously treated with CDK4/6 therapy, DCR was 45%.[27]
  - ✓ Zenocutuzumab/trastuzumab/vinorelbine in patients with HER2+ MBC previously treated with T-DM1: DCR at 6 months of 35%.[28]



Zenocutuzumab/trastuzumab/vinorelbine after T-DM1



25. Geuijen et al. Unbiased Combinatorial Screening Identifies a Bispecific IgG1 that Potently Inhibits HER3 Signaling via HER2-Guided Ligand BlockadeCancer Cell. 2018.
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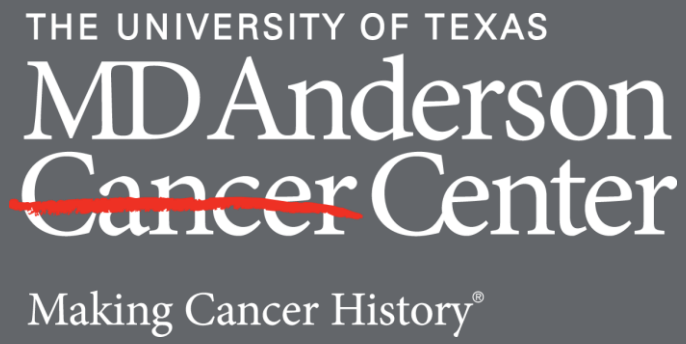
Conclusions

1. BsAbs are engineered to target different Ags/epitopes, with a variety of mechanisms of action.
2. Through engineering it is possible to design different structures and optimize desired molecular function for each clinical context.
3. Management of BsAbs toxicities requires specific expertise in clinical use.
4. Understanding mechanisms of resistance to BsAbs will be critical for developing rational combination regimens or sequencing with other therapeutics, to improve patient outcomes.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