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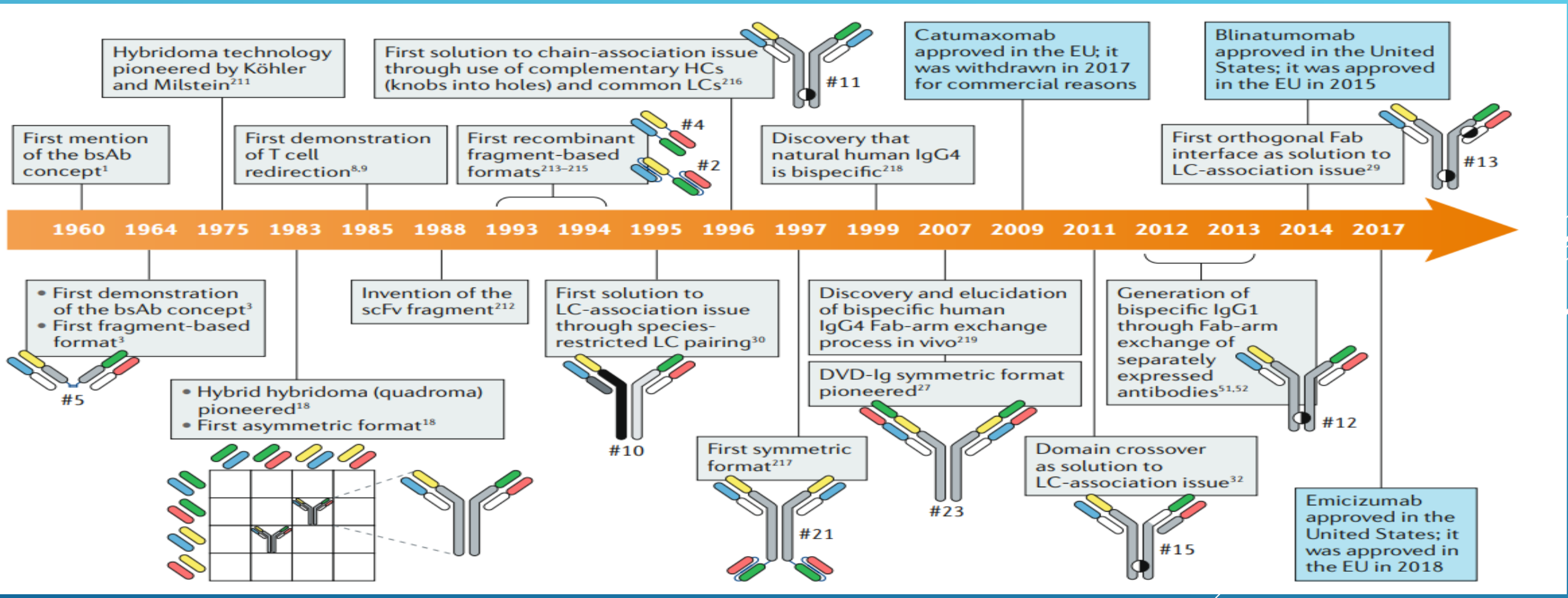
ΑΙΜΑΤΟΛΟΓΙΚΗ ΚΛΙΝΙΚΗ, ΠΑΓΝΗ

Bispecific Antibodies

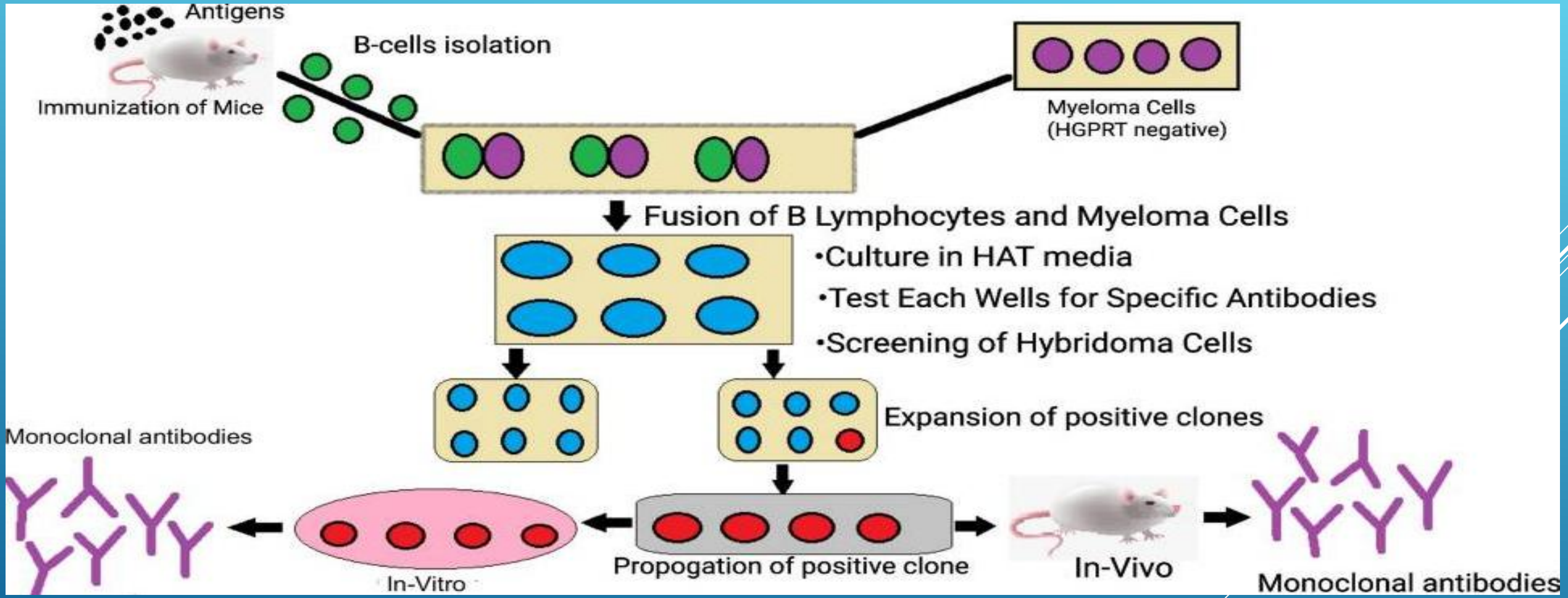
- Over the last three decades, therapeutic antibodies have become a key component of cancer treatment due to their specificity and sensitivity
- Excellent therapeutic effects of monoclonal antibodies (mAb) such as Rituximab (anti-CD20) and Trastuzumab (anti-HER2), which have been approved for the treatment of B-cell malignancies and breast cancer with promising results *(Coiffier et al., 2002)*
- A **bispecific antibody (bsAb)** is an artificial protein that can bind to two different types of antigens or two different epitopes on the same antigen
- The dual binding capability of bsAbs allows for synergistic antigen targeting, resulting in more complex mechanisms of action compared with conventional mAbs . This can prevent escape mechanisms seen with mAb treatment and increase selectivity therefore decreasing on target-off tumor effects *(Guidi et al., 2025)*

Development history of bsAb platforms



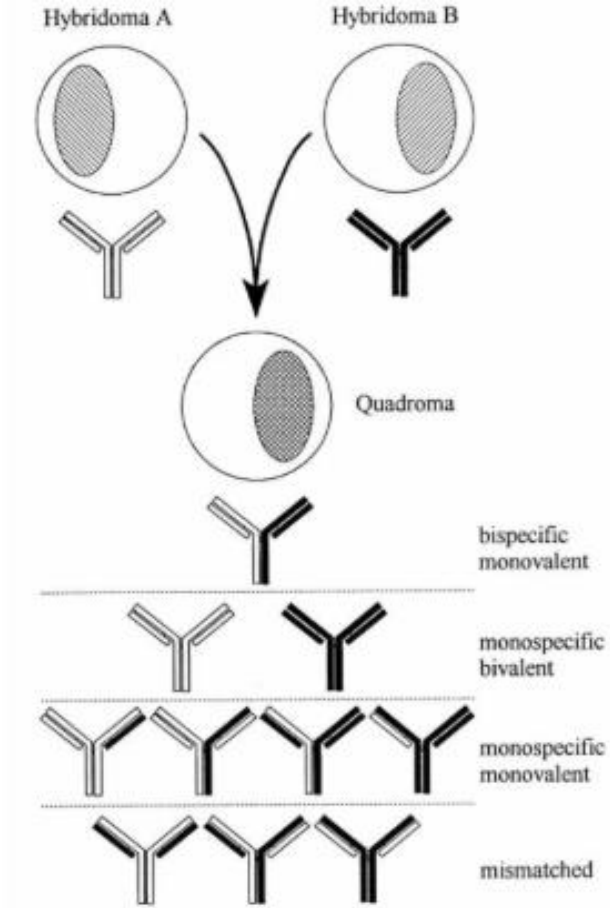
Adopted from: Labrijn, A. F., Janmaat, M. L., Reichert, J. M., & Parren, P. W. H. I. (2019). Bispecific antibodies: a mechanistic review of the pipeline. *Nature Reviews Drug Discovery*, *18*(8), 585–608.

Hybridoma technology



Adopted from: Mitra, S., & Tomar, P. C. (2021). Hybridoma technology; advancements, clinical significance, and future aspects. Journal of Genetic Engineering and Biotechnology, 19(1), 159.

Quadroma technology and the chain association problem



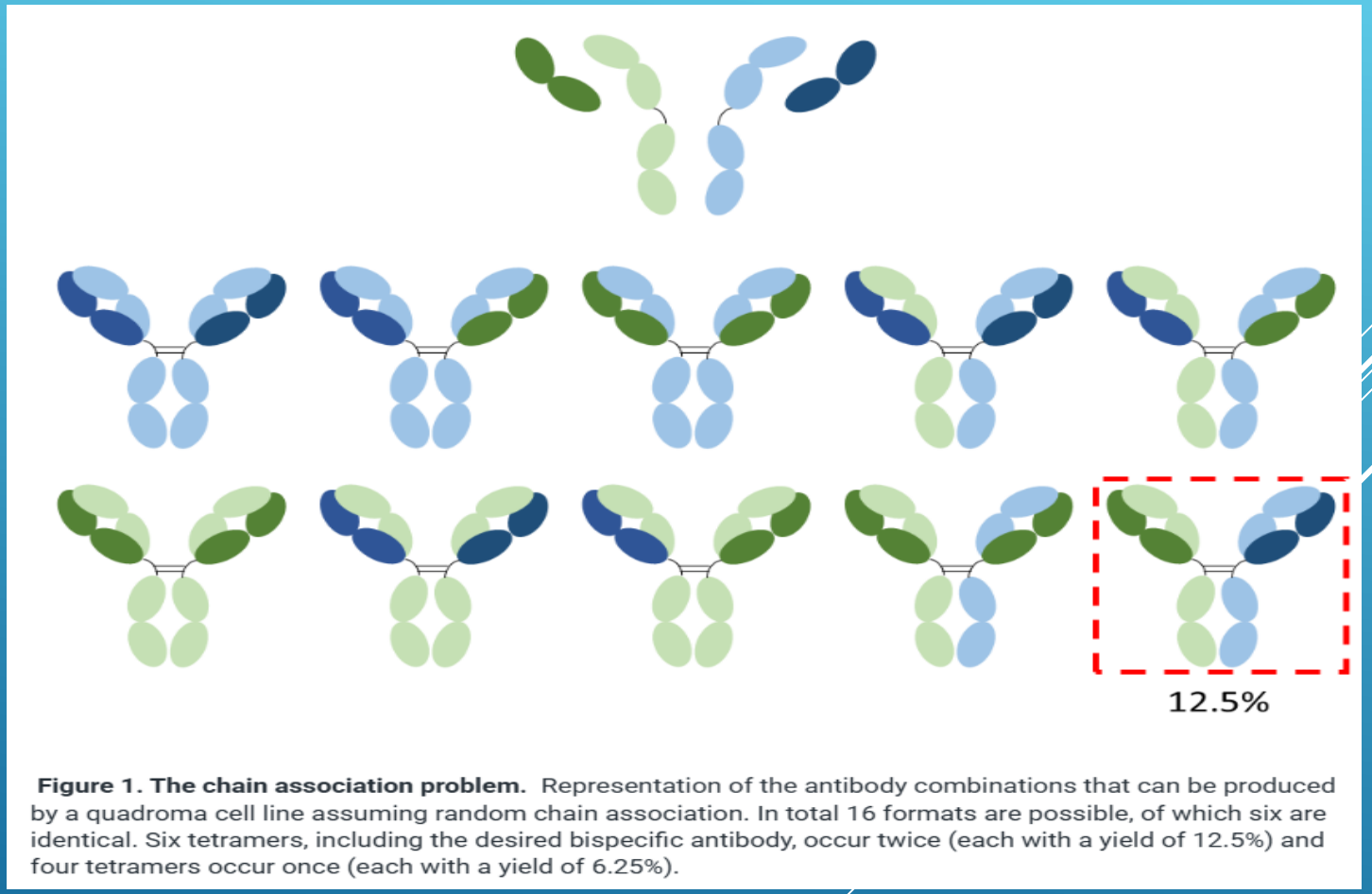
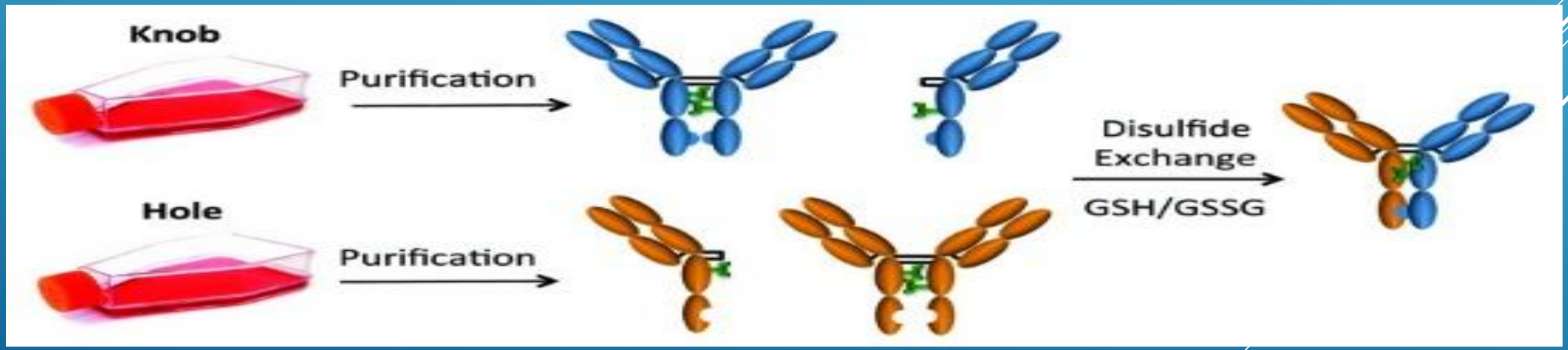


Figure 1. The chain association problem. Representation of the antibody combinations that can be produced by a quadroma cell line assuming random chain association. In total 16 formats are possible, of which six are identical. Six tetramers, including the desired bispecific antibody, occur twice (each with a yield of 12.5%) and four tetramers occur once (each with a yield of 6.25%).

Adopted from: Kroesen, B., Helfrich, W., Molema, G., & De Leij, L. (1998). Bispecific antibodies for treatment of cancer in experimental animal models and man.
Figure adopted from: https://absoluteantibody.com/antibody-resources/antibody-engineering/bispecifics/

Knobs into holes technology

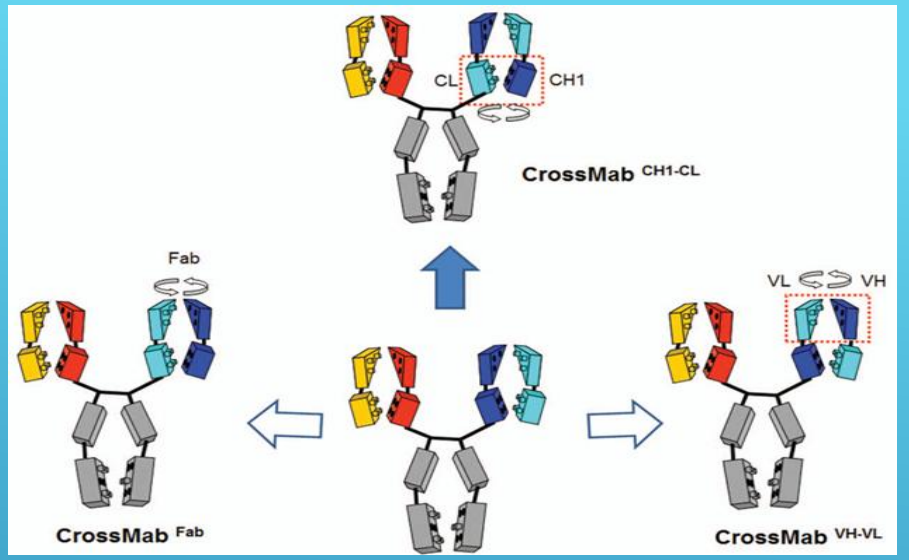
- A method of creating bispecific antibodies (bsAbs) where two heavy chains of antibodies are engineered to form a "knob" and a "hole" in their Fc regions
- The "knob" is formed by introducing a bulky amino acid (e.g., tyrosine) at a specific position in the heavy chain
- The "hole" is created by replacing a bulky amino acid with a smaller one at a different position in the heavy chain
- The knob and hole are designed to interact, promoting the formation of a stable heterodimer, which is the basis for the bispecific antibody



2013 Nov-Dec;5(6):872-81.
doi: 10.4161/mabs.26307. Epub 2013 Aug 29.

Light chain (LC) association problem

- The correct pairing of heavy chains from different antibodies was already achieved by knobs into holes technology
- However, using the knob-into-hole technology was just half of the molecular puzzle
- Multiple strategies have been developed to tackle this issue
- **Restricted LC pairing**
- **Common LC**
- **Dual-Acting Fab (DAF)-IgGs**
- **CrossMab technology**



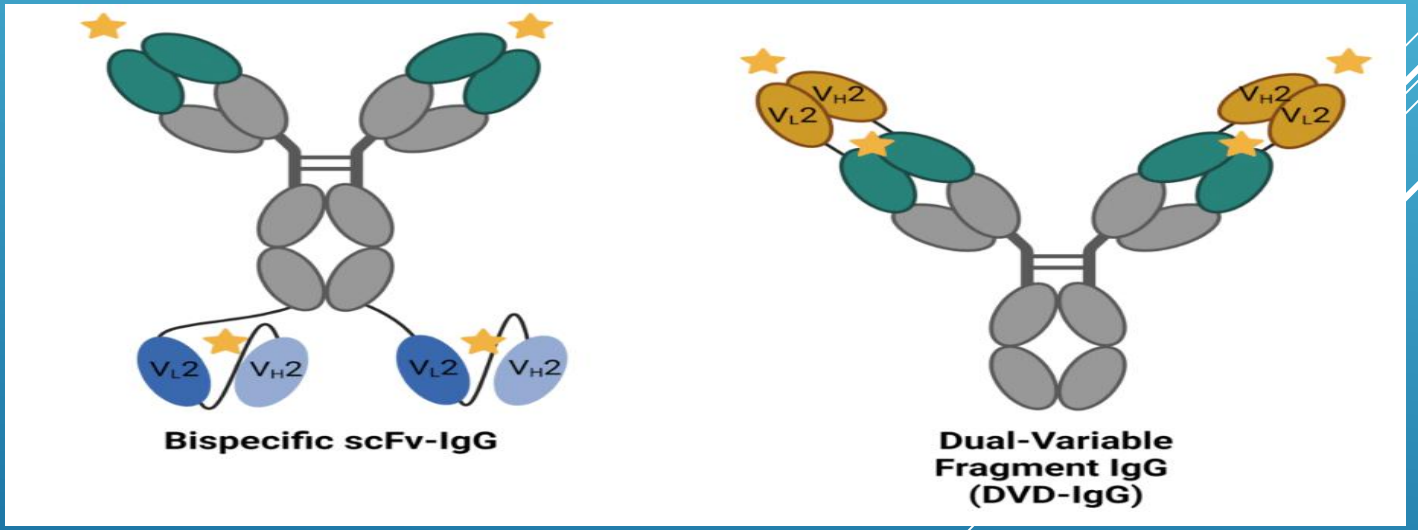
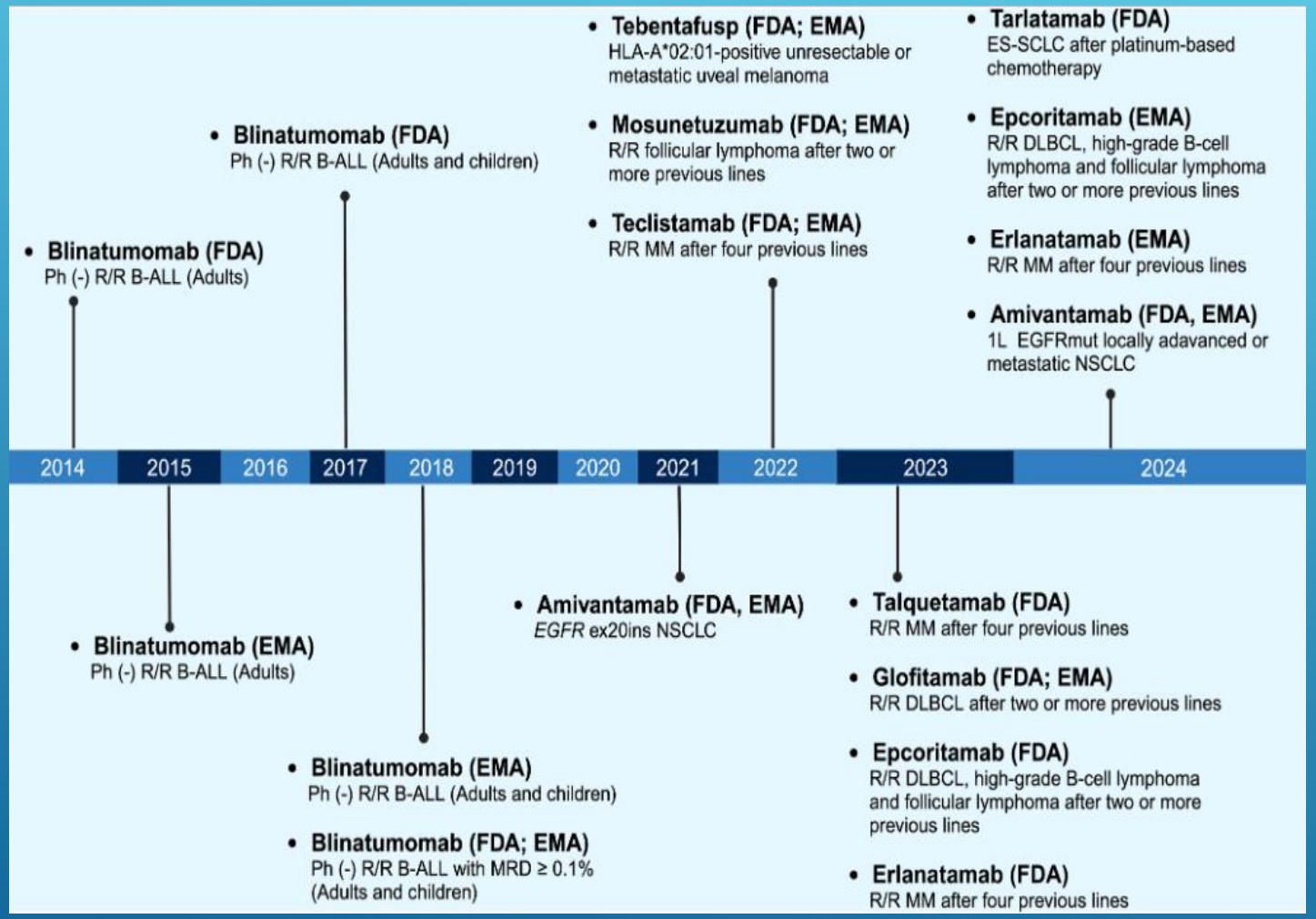
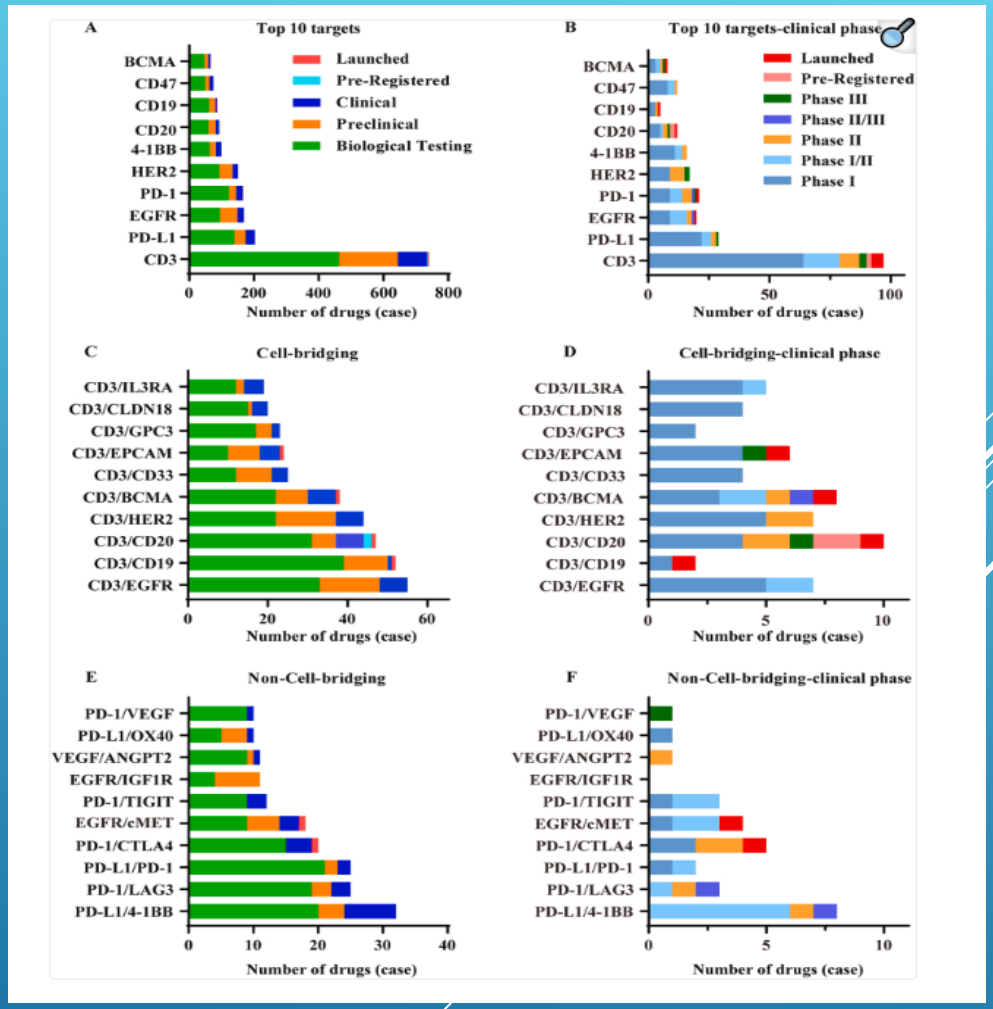


Figure adopted from: https://www.rapidnovor.com/bispecific-and-multispecific-antibodies/

Klein, C., Sustmann, C., Thomas, M., Stubenrauch, K., Croasdale, R., Schanzer, J., Brinkmann, U., Kettenberger, H., Regula, J. T., & Schaefer, W. (2012). Progress in overcoming the chain association issue in bispecific heterodimeric IgG antibodies. mAbs, 4(6), 653–663.

Global approval of bsAb drugs

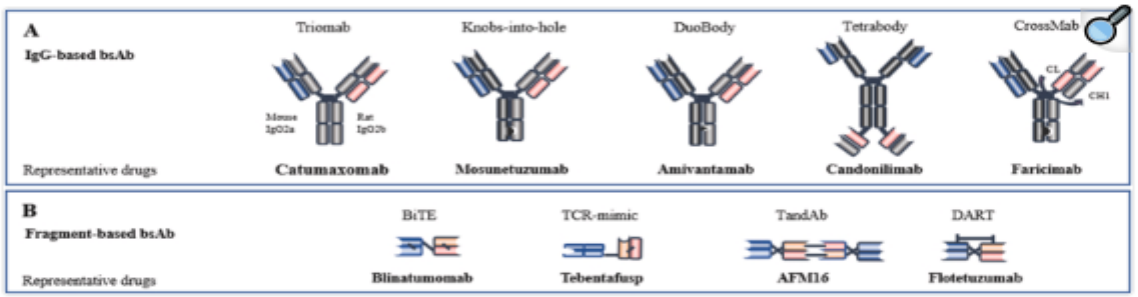




Guidi, L., Etessami, J., Valenza, C., Valdivia, A., Meric-Bernstam, F., Felip, E., & Curigliano, G. (2025c). Bispecific antibodies in hematologic and solid tumors: Current landscape and therapeutic advances. American Society of Clinical Oncology Educational Book, 45(3).
Sun, Y., Yu, X., Wang, X., Yuan, K., Wang, G., Hu, L., Zhang, G., Pei, W., Wang, L., Sun, C., & Yang, P. (2023). Bispecific antibodies in cancer therapy: Target selection and regulatory requirements. Acta Pharmaceutica Sinica B, 13(9), 3583–3597.

Bispecific formats

- bsAb drugs can be either fragment-based or IgG-based antibodies
- Fragment-based antibodies are easier to make but have low half-life
- IgG-based antibodies are similar in structure to native antibodies, and all have Fc regions, have increased half-life and multiple activities of bispecific antibodies, such as antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cell phagocytosis (ADCP) but are more difficult to make due to the chain associated issue



Representative bispecific antibodies and their format. According to the existence of the Fc region, bispecific antibodies can be divided into two categories: (A) IgG-based bsAbs and (B) Fragment-based bsAbs. BiTE, bispecific T-cell engager; TandAb, tandem diabody; DART, dual affinity retargeting.

Adopted from: Sun, Y., Yu, X., Wang, X., Yuan, K., Wang, G., Hu, L., Zhang, G., Pei, W., Wang, L., Sun, C., & Yang, P. (2023b). Bispecific antibodies in cancer therapy: Target selection and regulatory requirements. Acta Pharmaceutica Sinica B, 13(9), 3583–3597.

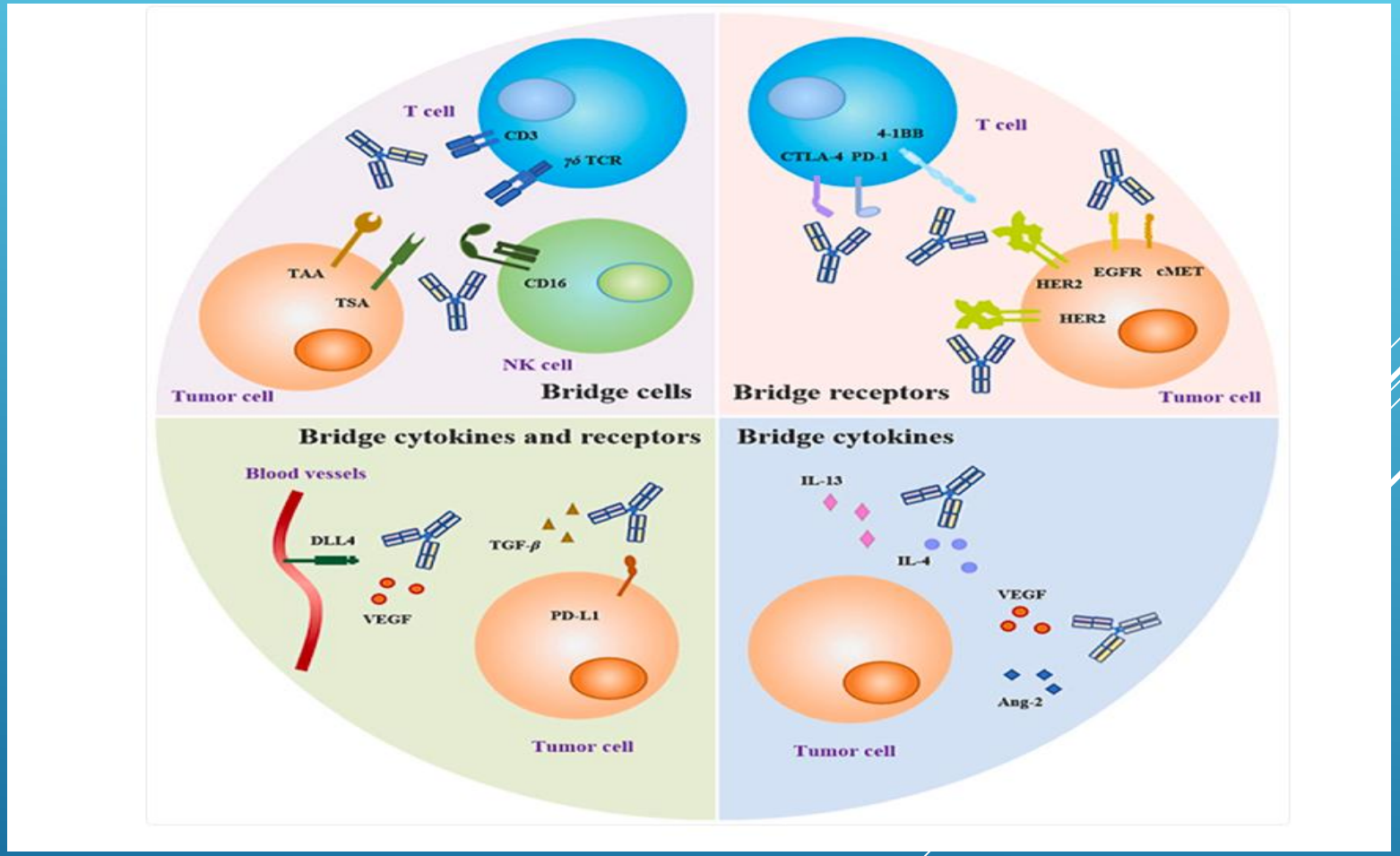
Affinity and Valency

- The **Affinity** of bispecific antibodies is a major factor influencing overall tolerability and efficacy
- For CD3-targeting T-cell engagers, the affinity of the CD3 arm is a key factor in the success of T-cell bispecific antibodies (T-bsAbs). The CD3 arm with too high affinity would lead to excessive release of cytokines and affect the tissue distribution of bsAbs, limiting their reach to the target site
- In one study, PSMA/CD3 bispecific antibodies with lower CD3 affinity were reported to be more effective in killing tumor cells and reducing the incidence and severity of cytokine release syndrome (CRS) in prostate cancer patients compared to bsAbs with high CD3 affinity
- Additionally, bispecific antibodies can achieve high selectivity against tumor cells by decreasing the affinity of arms to tumor-specific antigens (TSA). HER2 T-cell-dependent bispecific antibody (TDB) is a bsAb with two low-affinity HER2 arms. It has a strong binding ability to cells with high HER2 expression, while the binding rate to low HER2-expressing cells is low
- **Valency** refers to the number of binding sites in the antibody that can be used to bind antigens. It is another important factor in the design of bispecific antibodies, as it can affect the efficacy of the antibody
- Glofitamab is an example of a bsAb with a 2:1 valency against CD20 of B cells and CD3 of T cells. It has been shown to have 40-fold higher in vitro anti-tumor activity than 1:1 valency bsAbs

Slaga D., Ellerman D, Lombana N, Vij R, Li J, Hristopoulos M, et al. Avidity-based binding to HER2 results in selective killing of HER2-overexpressing cells by anti-HER2/CD3. Sci Transl Med. 2018;10:eaat5775. doi: 10.1126/scitranslmed.aat5775.
Bacac M., Colombetti S, Herter S, Sam J, Perro M, Chen S, et al. CD20-TCB with obinutuzumab pretreatment as next-generation treatment of hematologic malignancies. Clin Cancer Res. 2018;24:4785–4797.

Mechanism of action of bsAb

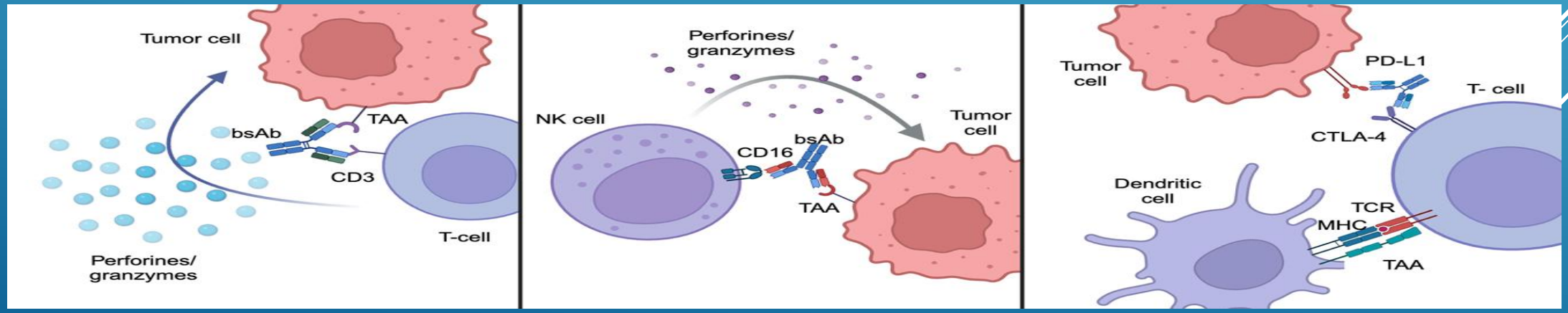
- According to National Medical Products Administration (NMPA) bispecific antibodies can be classified into four categories based on their mechanism of action: bridging cells, bridging receptors, bridging cytokines and bridging receptors and cytokines
- The strength of obligate bsAbs is their ability to unlock novel functionalities that require two binding specificities to be connected in the same molecule



Sun, Y., Yu, X., Wang, X., Yuan, K., Wang, G., Hu, L., Zhang, G., Pei, W., Wang, L., Sun, C., & Yang, P. (2023c). Bispecific antibodies in cancer therapy: Target selection and regulatory requirements. Acta Pharmaceutica Sinica B, 13(9), 3583–3597.

Bridging cells

- Bispecific TCEs connect native CD4+ and CD8+ T cells with tumor cells by simultaneous binding to the CD3ε subunit of the T-cell receptor (TCR) complex and a specific Tumor Associated Antigen (TAA). This specific interaction determines T-cell activation, release of granzymes and perforins, and tumor cell lysis
- Outstanding results in many hematological malignancies and thus half of the bsAbs undergoing evaluation in trials are bsTCEs
- Other bsAbs bridging cells are NK-cell engagers bsAbs bind CD16 on NK cells, leading to tumor cell killing using perforins and granzymes
- bsAbs targeting PD-L1 and CTLA-4 enhance T-cell activation, while TAA binding promotes dendritic cell–mediated antigen presentation



Guidi, L., Etessami, J., Valenza, C., Valdivia, A., Meric-Bernstam, F., Felip, E., & Curigliano, G. (2025d). Bispecific antibodies in hematologic and solid tumors: Current landscape and therapeutic advances. American Society of Clinical Oncology Educational Book, 45(3).

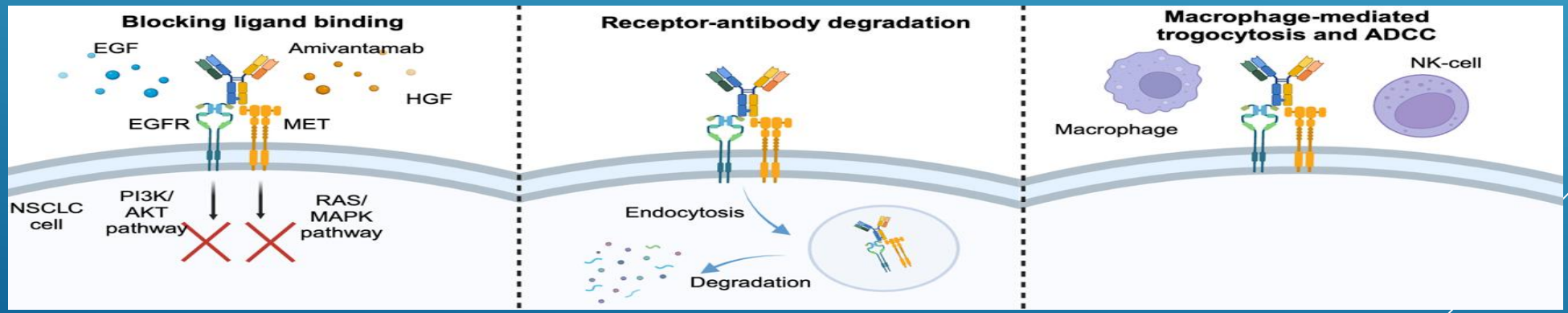
Dual Checkpoint Inhibitors

- Dual CPI-blocking bsAbs simultaneously target immune checkpoint receptors by binding 1 receptor (eg, PD-1, CTLA-4, LAG-3, or TIGIT) on the surface of a T cell with one arm, while the other arm engages a second immune checkpoint receptor. This second receptor can be located on the same T cell, tumor cell, or antigen-presenting cell
- Selective targeting of TAAs and checkpoint proteins could be effective in minimizing autoimmune adverse events
- The simultaneous binding of two checkpoint proteins has the potential to improve efficacy, boost immune responses, and overcome resistance mechanisms
- Numerous drugs are in trials or under development combining anti-PD-(L)1 blockade with anti-CTL-4, LAG-3, TIM-3
- Furthermore the development of anti-TGFβ × PD-L1 bsAbs introduces an innovative strategy to simultaneously inhibit multiple immunosuppressive pathways in the TME, enhancing the immune system's ability to combat cancer

Zhang T, Lin Y, Gao Q: Bispecific antibodies targeting immunomodulatory checkpoints for cancer therapy. Cancer Biol Med 20:181-195, 2023

Dual receptor inhibition bsAbs

- bsAbs structure is designed to target two different TAAs simultaneously, potentially limiting resistances to a single-pathway modulation, inherent in the plasticity of cancer cells. Moreover, dual receptor inhibition by bsAbs may reduce the incidence of on-target off-tumor toxicities, owing to the need to engage to both receptors to carry out their cytotoxic effect
- Several bsAbs designed for dual receptor inhibition are being evaluated in clinical trials worldwide. The first one approved for patients with solid tumors was the anti-EGFRxMET bsAb amivantamab
- This agent was approved for patients with non–small cell lung cancer (NSCLC) harboring EGFR exon 20 insertion, which represents the third most common EGFR mutation type, resulting in altered TKI binding and intrinsic resistance to EGFR TKIs
- Among other TAAs explored for dual signaling inhibitors, one of the major areas of development is represented by the HER2, whose overexpression is a major driver in various solid tumors (zanidatamab, zenocutuzumab)



Guidi, L., Etessami, J., Valenza, C., Valdivia, A., Meric-Bernstam, F., Felip, E., & Curigliano, G. (2025d). Bispecific antibodies in hematologic and solid tumors: Current landscape and therapeutic advances. American Society of Clinical Oncology Educational Book, 45(3).

Bispecific T-cell Enganger (BiTE) therapy in hematological malignancies

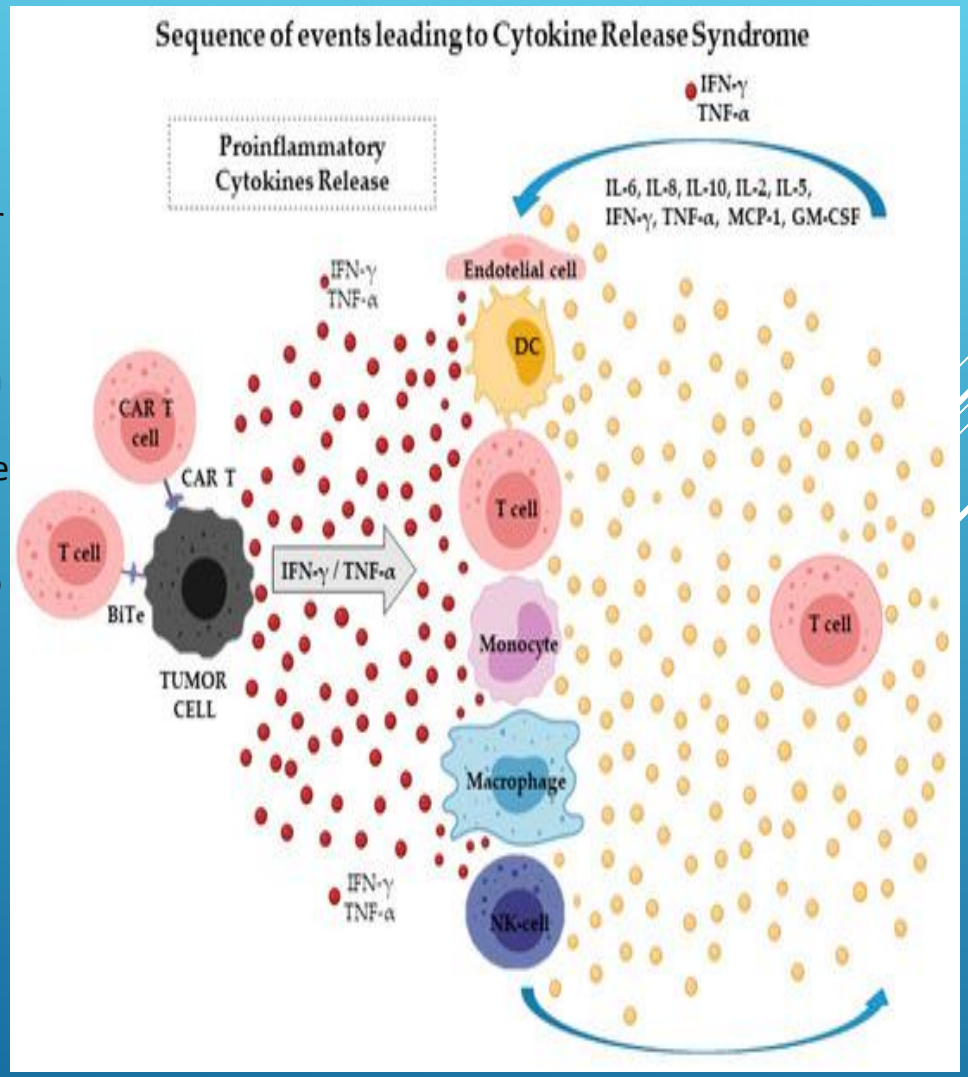
- Several bispecific antibodies have been approved by the FDA for the treatment of non-Hodgkin lymphoma (NHL), particularly for relapsed or refractory diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL). These include anti-CD20 bsTCE epcoritamab (Epkinly), glofitamab (Columvi), and mosunetuzumab (Lunsumio)
- Three bispecific antibodies have received FDA approval for treating relapsed or refractory multiple myeloma (RRMM): These include anti-BCMA bsTCE teclistamab (Tecvayli) and elranatamab (Elrexfio), and anti-GPRC5D bsTCE talquetamab (Talvey)
- Blincyto (blinatumomab) is an anti-CD19 bsTCE immunotherapy drug used to treat B-cell precursor acute lymphoblastic leukemia (ALL) in adults and children
- bsAbs have shown remarkable success in hematologic malignancies with very good overall response in heavily treated patients , who would otherwise have a dismal prognosis, with a manageable profile of toxicities

- Blinatumomab is a bispecific T cell–engager antibody construct that directs T cells to CD19+ cells. It is indicated in relapsed Bcell precursor-ALL and in first remission in patients with Minimal Residual Disease (MRD) positive
- Treatment with blinatumomab resulted in significantly longer overall survival than chemotherapy among adult patients with relapsed or refractory B-cell precursor ALL
- In MRD positive B-cell ALL patients, 80% achieved MRD negativity with blinatumumab and thus having longer Overall Survival (OS) and higher Relapse-Free Survival at 18 months
- In Relapsed Multiple Myeloma Patients, who have received at least 3 prior treatment lines, BiTE therapy have an Overall Response rate of 60-70%
- Three-year follow-up of mosunetuzumab in R/R FL after ≥2 prior therapies showed Overall Response Rates (ORR) of 80%, long-lasting remissions and meaningful survival outcomes *(LH Sehn et al. Blood (2025) 145 (7): 708–719.)*
- Anti-CD20xCD3 BsAb Glofitamab and Epcoritamab in Relapsed/Refractory DLBCL patients results in 40% Complete Response (CR) and 60% ORR *(Falchi L. et al. Blood (2023) 141 (5): 467–480.)*

Kantarjian et al. N Engl J Med 2017;376:836-847 , Gökbuget et al. Blood (2018) 131 (14): 1522–1531.
Moreau P et al. N Engl J Med. 2022;387:495. 2 , Ajai C et al. N Engl J Med 2022;387:2232-2244

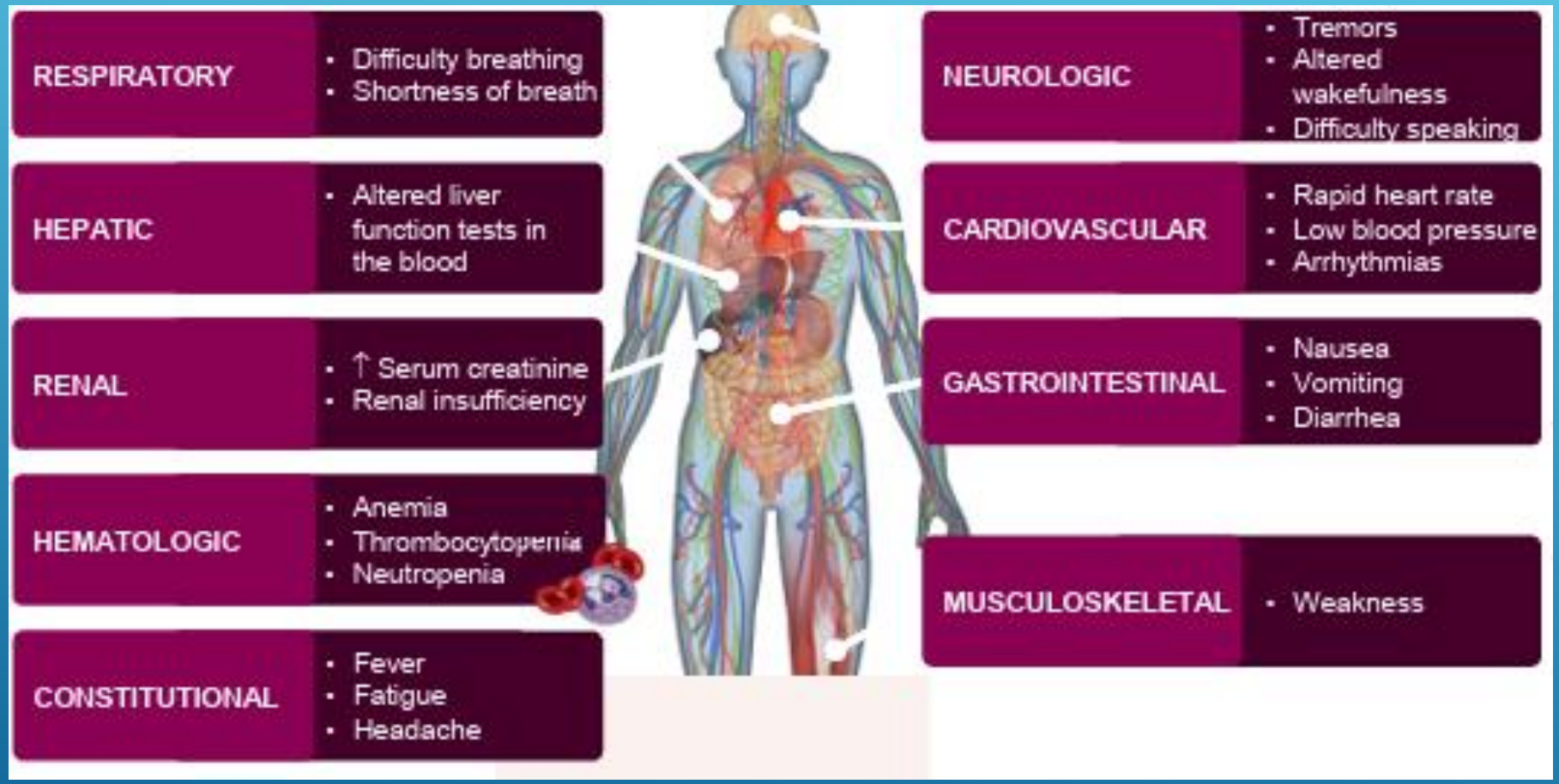
BiTE therapy Side Effects – CRS/ICANS

- Cytokine release syndrome (CRS) is an acute systemic inflammatory syndrome characterized by fever and multiple organ dysfunction. CRS is most often associated with chimeric antigen receptor (CAR)-T cell therapy, but it also occurs in association with bispecific T cell engager therapy
- CRS is a supraphysiologic response to immune therapy that activates or engages T cells and/or other immune effector cells. The systemic reaction is associated with increased levels of inflammatory cytokines and activation of T lymphocytes, macrophages, and endothelial cells
- Cytokines contribute importantly to the pathophysiology and clinical manifestations of CRS. In the setting of T cell-engaging immunotherapies, CRS is triggered by release of interferon gamma (IFN-g) by activated T cells or tumor cells. IFN-g activates macrophages, which produce excessive interleukin (IL)-6, tumor necrosis factor alpha (TNF-a), and IL-10
- IL-1, IL-5, IL-8, IL-10, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are also consistently elevated in CRS and may also contribute to the pathophysiology of CRS.
- Immune effector cell-associated neurotoxicity syndrome (ICANS) is a neuropsychiatric syndrome that can occur in some patients who are treated with immunotherapy, which may or may not accompany CRS
- It is thought that systemic inflammation and high levels of circulating cytokines result in endothelial cell activation and blood-brain barrier (BBB) disruption, which in turn causes an inflammatory cascade within the central nervous system (CNS), subsequent alterations in cortical and subcortical function, and diffuse cerebral edema in some cases.



UpToDate. (n.d.). UpToDate. https://www.uptodate.com/contents/cytokine-release-syndrome-crs?search=crs&source=search_result&selectedTitle=1~82&usage_type=default&display_rank=1

BiTE therapy Side Effects – CRS/ICANS

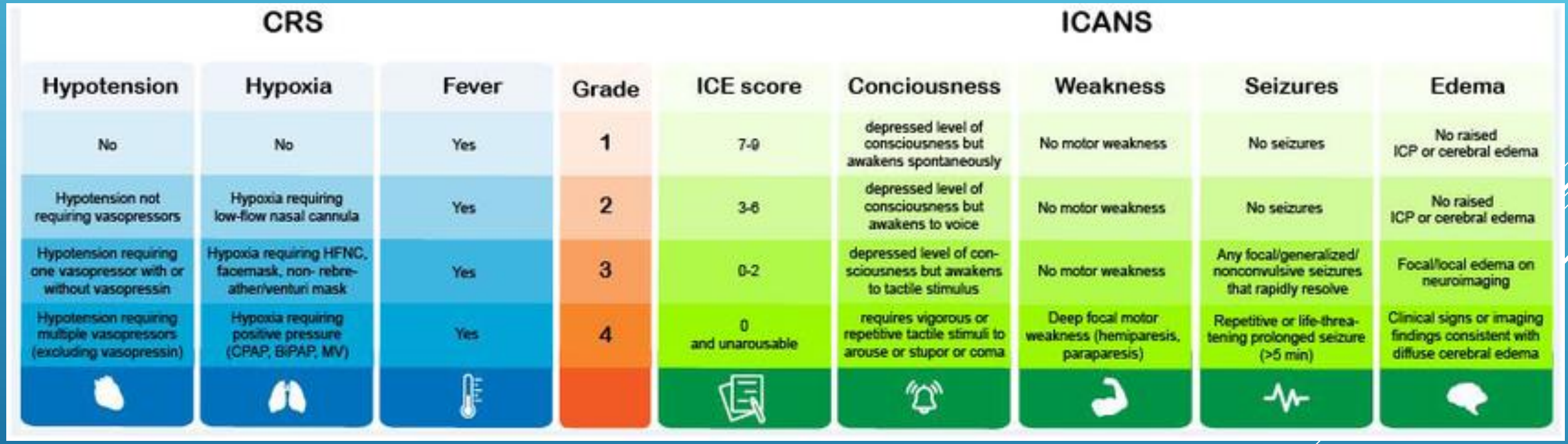


Mitigation and monitoring for CRS

- Step-up dosing with hospitalization for monitoring
- Frequent vital signs
- Rule out infection
- Laboratory monitoring
- Early intervention with tocilizumab

Oluwole OO, Davila ML. J Leukoc Biol. 2016;100:1265. June CH, et al. Science. 2018;359:1361. Brudno JN, Kochenderfer JN. Blood. 2016;127(26):3321. Brudno JN, Kochenderfer JN. Blood Rev. 2019:34:45. Shimabukuro-Vornhagen, et al. J Immunother Cancer. 2018;6:56. Lee DW, et al. Biol Blood Marrow Transplant. 2019;25:625.

BiTE therapy Side Effects – CRS/ICANS



CRS				ICANS				
Hypotension	Hypoxia	Fever	Grade	ICE score	Conciousness	Weakness	Seizures	Edema
No	No	Yes	1	7-9	depressed level of consciousness but awakens spontaneously	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension not requiring vasopressors	Hypoxia requiring low-flow nasal cannula	Yes	2	3-6	depressed level of consciousness but awakens to voice	No motor weakness	No seizures	No raised ICP or cerebral edema
Hypotension requiring one vasopressor with or without vasopressin	Hypoxia requiring HFNC, facemask, non- rebre-athen/venturi mask	Yes	3	0-2	depressed level of con-sciousness but awakens to tactile stimulus	No motor weakness	Any focal/generalized/ nonconvulsive seizures that rapidly resolve	Focal/local edema on neuroimaging
Hypotension requiring multiple vasopressors (excluding vasopressin)	Hypoxia requiring positive pressure (CPAP, BiPAP, MV)	Yes	4	0 and unarousable	requires vigorous or repetitive tactile stimuli to arouse or stupor or coma	Deep focal motor weakness (hemiparesis, paraparesis)	Repetitive or life-threa-tening prolonged seizure (>5 min)	Clinical signs or imaging findings consistent with diffuse cerebral edema

- Grading for CRS in CAR-T therapy is according to the criteria of the American Society for Transplantation and Cellular Therapy (ASTCT), whereas CRS caused by other types of immune intervention like bsAb are graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0, although these two grading systems have minimal differences

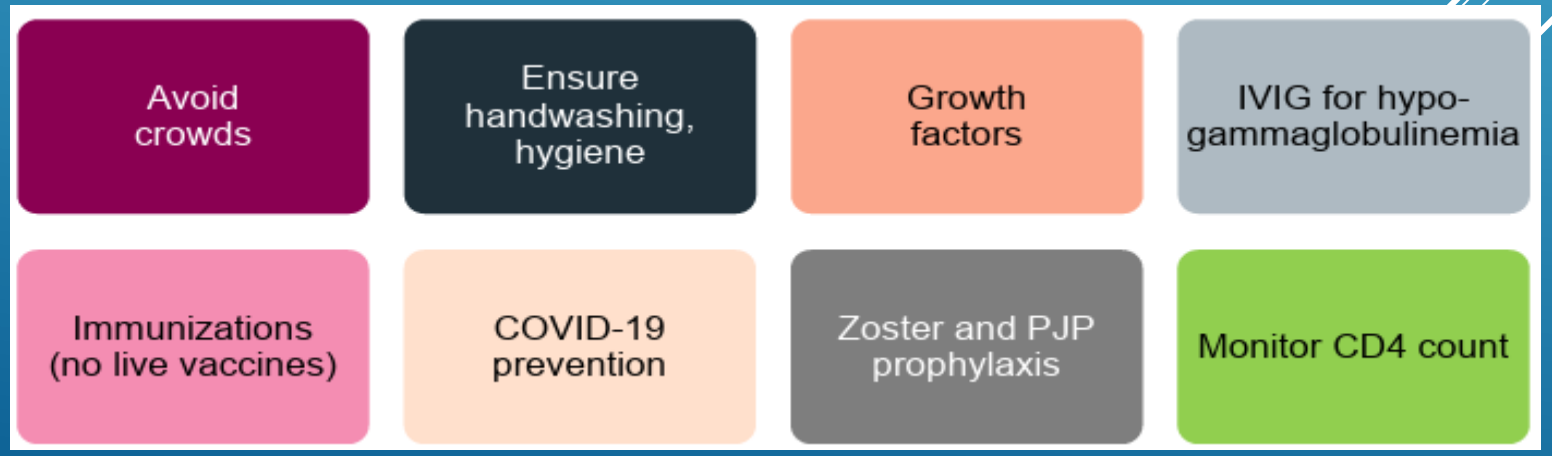
Shimabukuro-Vornhagen, Alexander & Böll, Boris & Schellongowski, Peter & Valade, Sandrine & Metaxa, Victoria & Azoulay, Elie & von Bergwelt, Michael. (2021). Critical care management of chimeric antigen receptor T-cell therapy recipients. CA: A Cancer Journal for Clinicians. 72. 10.3322/caac.21702.

BiTE therapy Side Effects – CRS/ICANS

- BsAbs appear to be associated with a lower incidence and severity of key toxicities such as CRS and neurotoxicity than CAR T-cell therapy and with an earlier onset of CRS and with a shorter duration *(Crombie J. et al. Blood (2024) 143 (16): 1565–1575.)*
- There are currently no BsAb-specific consensus guidelines, and guidance is typically modeled after recommendations developed for chimeric antigen receptor (CAR) T-cell therapy without taking into account key differences between the toxicity profiles of these classes of drugs *(D.W.Leeetal./BiolBloodMarrowTransplant25(2019)625 638)*
- Generally CRS occurs in 30-50% of patients receiving BiTE therapy, most of them being Grade I or II. Grade 3 CRS occurs in <5%
- Overall, ICANS-like toxicity, including delirium, dysgraphia, tremor, lethargy, difficulty concentrating, etc, was rare (1%-8%) across studies
- For CRS mitigation all of the BiTE products have a ramp-up dosing schedule, premedication with corticosteroids and patients are hospitalized for 24-48 h after the dose or they are they remain near the hospital. If CRS occurs dexamethasone and tocilizumab are mostly used with good outcomes and no impact in treatment

BiTE therapy Side Effects - Infections

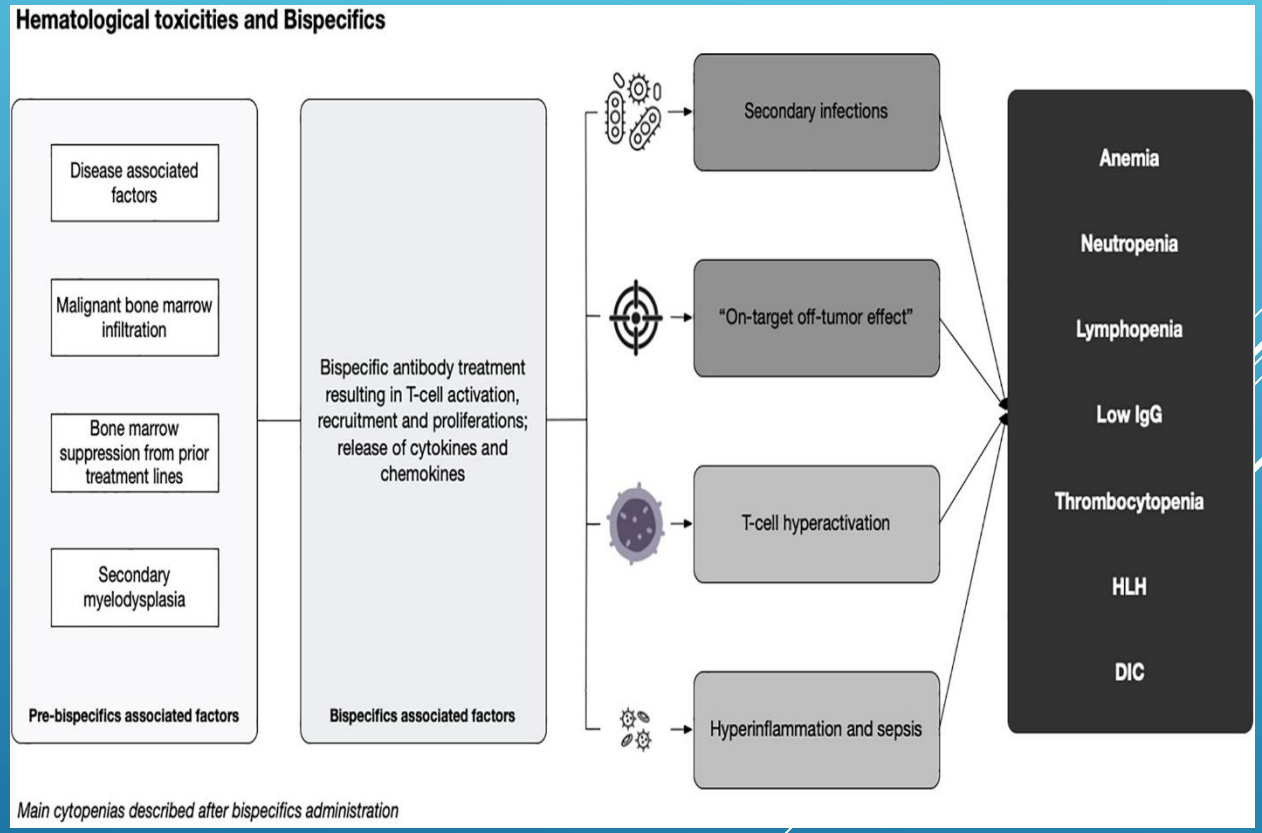
- Infection rates with BiTE therapy in hematological malignancies are common
- In NHL patients infection rates are around 40%, with 15% being Grade 3 requiring hospitalization
- In Multiple Myeloma (MM) patients infection rates are higher with anti-BCMA bsAb. Around 75% of patients experience an infection, mosty Respiratory tract infections and around 30% of patients have Grade 3 infection
- Infections in MM patients is multifactorial. Some of the causes include hypogammaglobulinemia , lymphopenia, defective Th1 response. MM patients are also usually more heavily treated patients than NHL patients
- Recommendations for minimizing infection risk in patients receiving BiTE therapy include proper vaccination status, monitoring for infections and reactivation of viruses like CMV or Adenovirus, prophylaxis for herpes zoster and Pneumocystis jirovecii, appropriate use of G-SCF in neutropenic patients and Ig replacement if indicated *(Raje N. et al. Blood Cancer Journal volume 13, Article number: 116 (2023))*



Aumann S. et al. Blood (2024) 144 (Supplement 1): 5124.
www.thelancet.com/haematology Vol 9 February 2022

BiTE therapy Side Effects - Cytopenias

- Hematological toxicities are common and potentially harmful with BiTE therapy
- There is a paucity of data on common hematological toxicities observed in the context of BsAbs
- Some of these toxicities are well characterized as “on-target, off-tumor” effects of the T-cell bispecifics, particularly the profound lymphopenia and hypogammaglobulinemia
- The reasons for these phenomena are multifactorial and associated with the disease (which is often bone marrow based), previous therapies (most patients are heavily pretreated), the patient's immune repertoire, and the effect of the individual bispecific molecule in inflammation, B-cell/plasma cell depletion and T-cells exhaustion. Production of cytokines by the bone marrow environment may also affect hematopoiesis during BiTE and BsAb therapy
- No clear recommendations can be made regarding eryhtropoietin and thrombopoitin agonists. Transfusions should be administered according to clinical indication
- G-CSF in case of neutropenia <1000/mm3 while avoiding dosing delays



De Assis, L. H., Fassi, D. E., & Hutchings, M. (2023). Bispecific antibody therapies. Hematology, 2023(1), 216–222.

Conclusion

- bsAbs are reshaping the pharmacopeia of solid tumors, hematologic malignancies and other non-malignant conditions
- Their dual-binding properties enhance the therapeutic efficacy of monoclonal antibodies, disrupting multiple signaling cascades simultaneously, preventing escape mechanisms and improving immune cell recruitment strategies
- Although bsAbs have shown remarkable success in hematologic malignancies, their expansion into solid tumors faces key challenges, including tumor heterogeneity, limited tumor penetration, and the risk of on-target, off-tumor toxicities. Addressing these challenges requires innovative engineering strategies, optimized delivery mechanisms, and careful patient selection to maximize therapeutic benefit while mitigating adverse effects
- There are ongoing trials examining of bsAb in earlier treatment lines and combining these drugs with other common chemotherapy, immunotherapy, checkpoint inhibitors and even combining two different bsAbs
- bsAbs provide exciting opportunities for novel drug design and development

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ΣΧΟΛΕΙΟ ΒΑΣΙΚΗΣ ΑΝΟΣΟΛΟΓΙΑΣ ΓΙΑ ΚΛΙΝΙΚΟΥΣ

HOTEL IBIS STYLES
HERAKLION CENTRAL