AbtBioConsult

Supporting biologics CMC development

Bispecifics: Different Formats bring Different Treatment Opportunities

But also Different CMC Challenges

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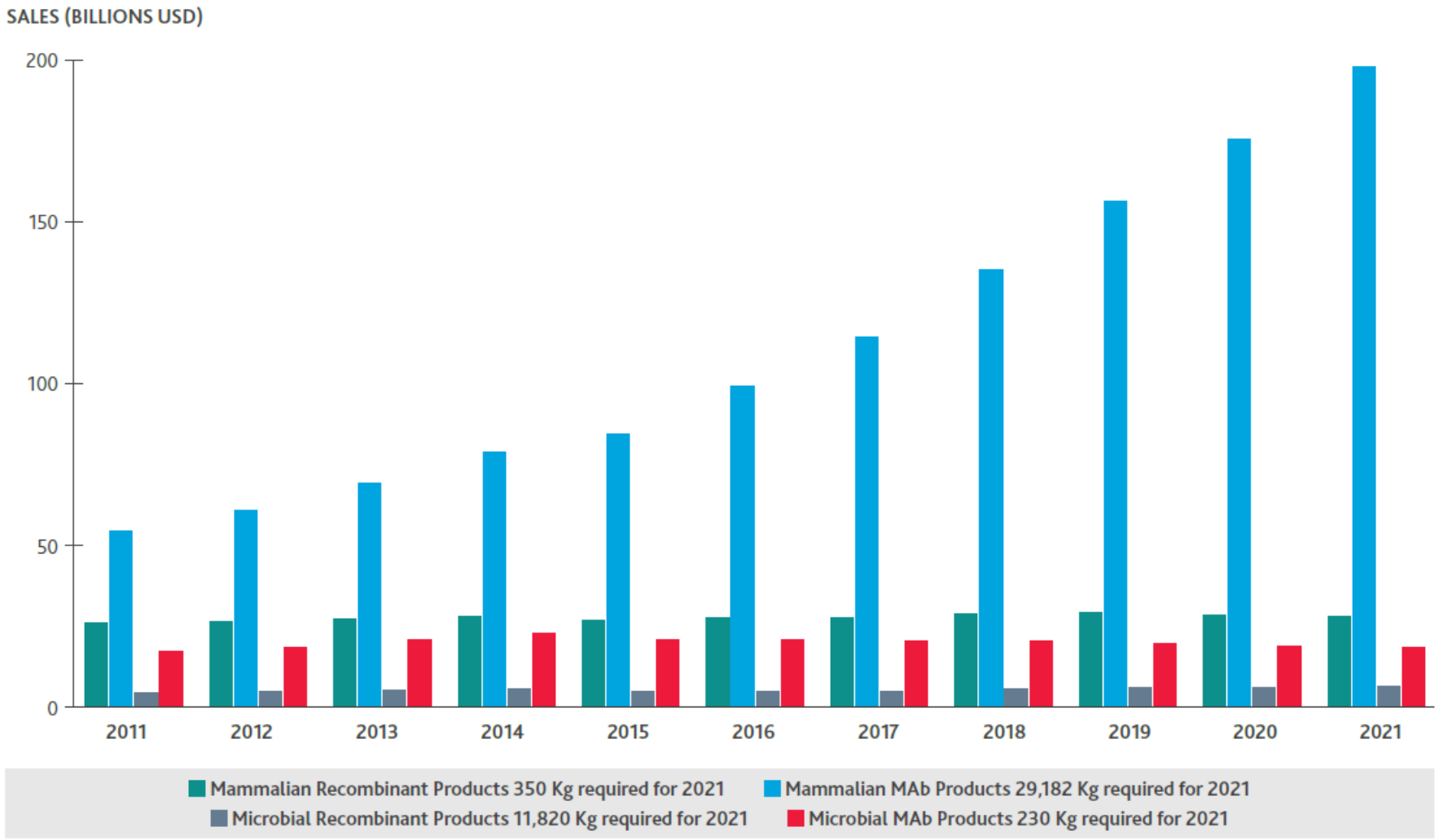
Agenda

1. Biopharmaceutical market trends for bispecifics
2. CMC challenges
3. Bispecifics case studies

Growth and Distribution of the Biopharmaceutical Market



Data and graphs derived from BDO's BPTG bioTRAK® database:
https://www.bdo.com/industries/life-sciences/bioprocess-technology

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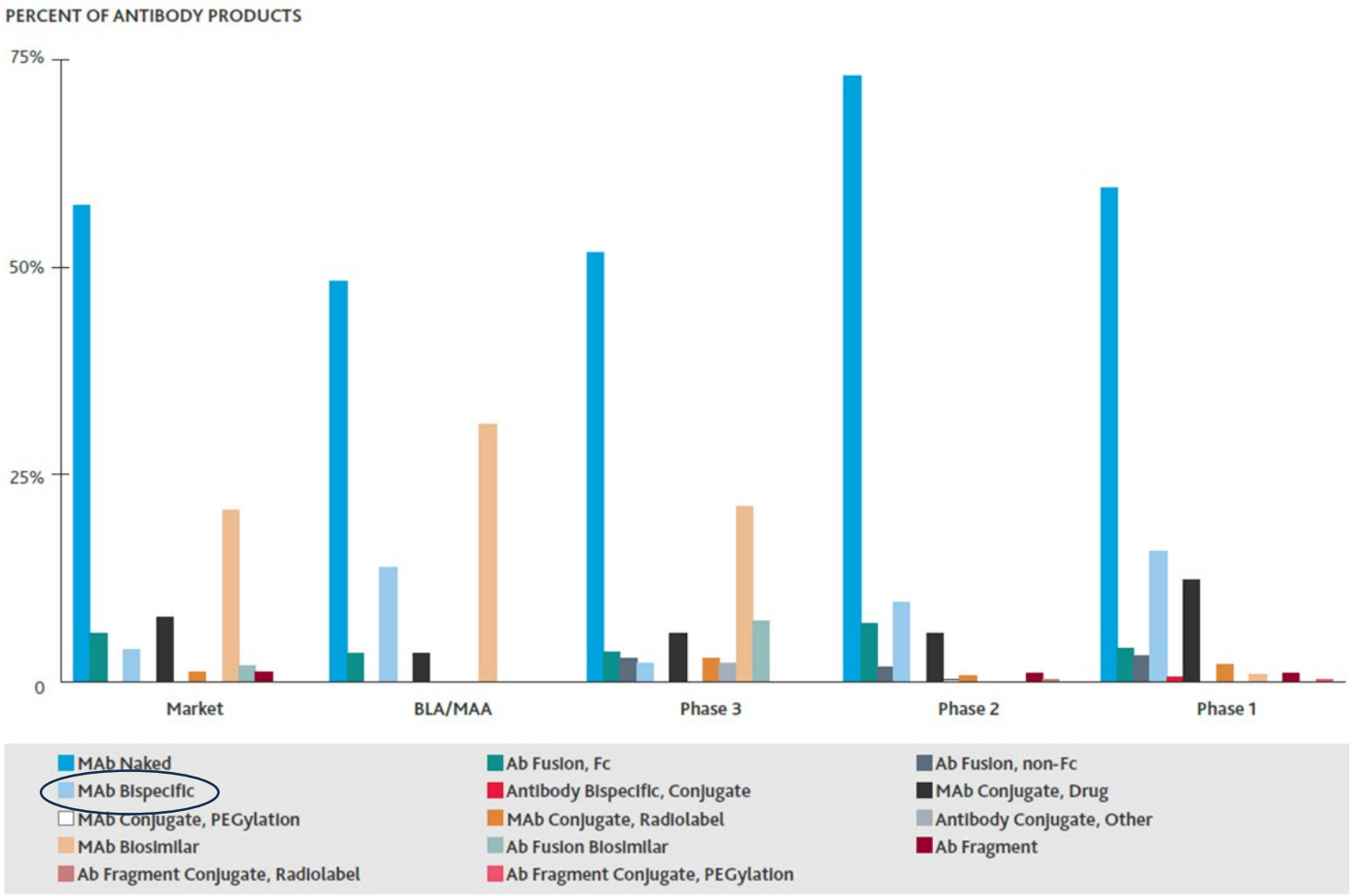
Mammalian Biologics Product Distribution by Product Type and Clinical Phase



Across the entire pipeline, naked mAbs are the dominant product type:

- Marketed Products: 57%
- BLA/MAA: 48%
- Phase 3: 52%
- Phase 2: 73%
- Phase 1: 60%

Bispecific mAb products constitute the following proportions of the MAb pipeline:

- Marketed Products: 4%
- BLA/MAA: 14%
- Phase 3: 2%
- Phase 2: 10%
- Phase 1: 17%

Data and graphs derived from BDO's BPTG bioTRAK® database:
https://www.bdo.com/industries/life-sciences/bioprocess-technology

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Bispecifics Approved or in Clinical Trials

14 bi-specific molecules approved for market by 2022

- 10 are structured as full-length antibodies
- 1 TCR-scFv fusion format
- 1 nanobody format
- 1 tandem scFv format

9 are targeting CD3 along with another target

- CD20, CD19, BCMA, gp100,

Bi-specifics in clinical trials

- >110 by 2021
- Formats: IgG-like and non-IgG like (no Fc region)

FDA-Approved Bispecific Antibodies

Trade Name	Active Ingredient	Year Approved	Indication
Blincyto	blinatumomab	2014	To treat Philadelphia chromosome-negative relapsed or refractory B cell precursor acute lymphoblastic leukemia
Hemlibra	emacizumab-kxwh	2017	To prevent or reduce the frequency of bleeding episodes in hemophilia A with factor VIII inhibitors
Rybrevant	amivantamab-vmjw	2021	To treat locally advanced or metastatic non-small cell lung cancer with certain mutations
Kimmtrak*	tebentafusp-tebn	2022	To treat a form of unresectable or metastatic uveal melanoma
Vabysmo	faricimab-svoa	2022	To treat neovascular (wet) age-related macular degenerated and diabetic macular edema
Tecvayli	teclistamab-cqyv	2022	To treat relapsed or refractory multiple myeloma
Lunsumio	mosunetuzumab-axgb	2022	To treat relapsed or refractory follicular lymphoma
Epkinly	epcoritamab-bysp	2023	To treat relapsed or refractory diffuse large B-cell lymphoma
Columvi	glofitamab-gxbm	2023	To treat relapsed or refractory diffuse large B-cell lymphoma or large B-cell lymphoma

Recent EMA approval of Talvey/Talquetamab (July 2023)

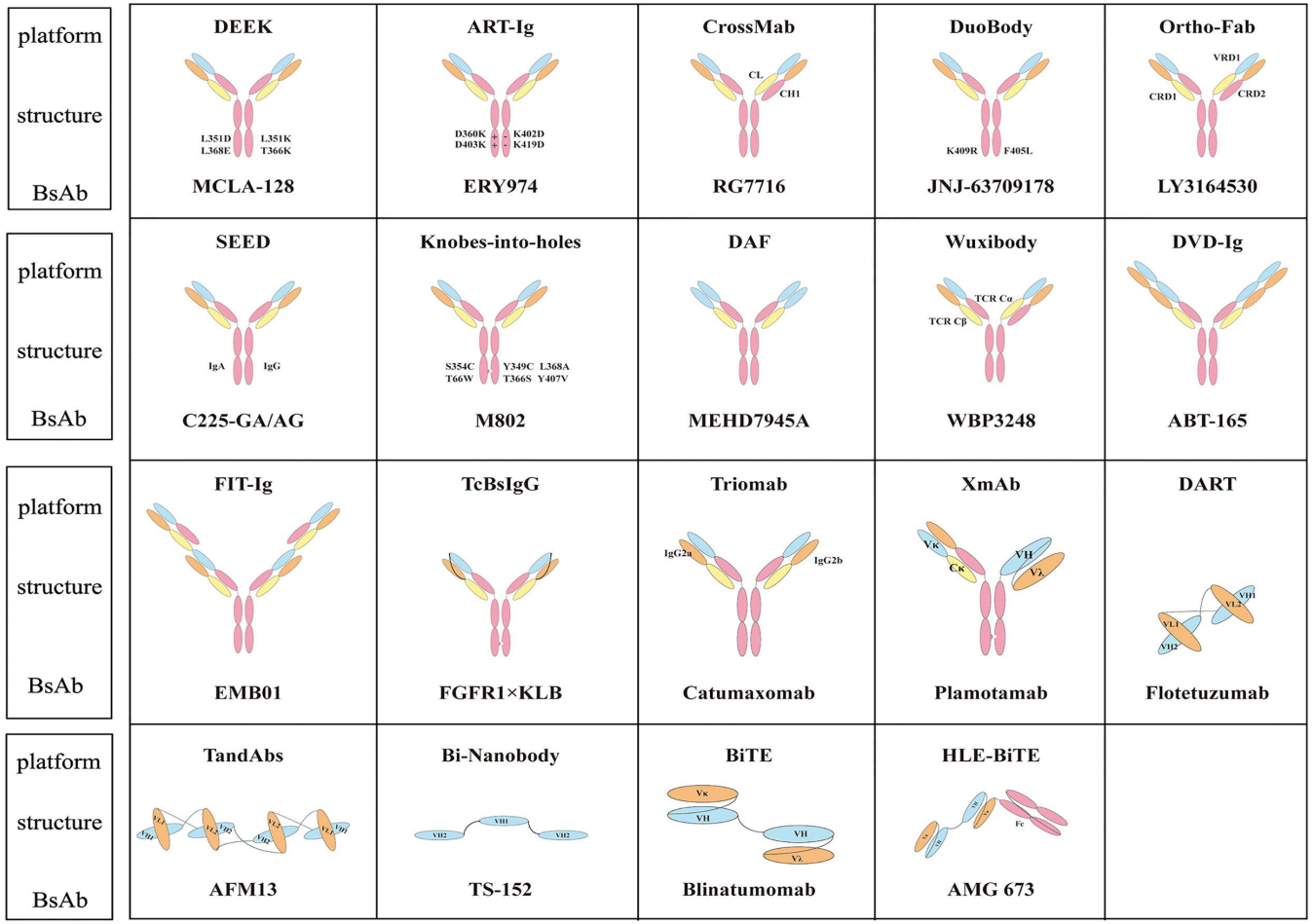
Bispecifics Anticipated Challenges

- Immunogenicity
  - non-natural molecules

CMC

- IgG vs non-IgG format
- Folding and pairing
  - One or more chains in expression vector
  - Fraction of non-correct formats
  - Homodimers vs heterodimers
- Aggregation tendency
- Titer and yield
- Product quality assessment
- Stability

Format matters!



Source: Bispecific Antibodies: From Research to Clinical Application (2021), Front. Immunol., J. Ma et al https://www.frontiersin.org/articles/10.3389/fimmu.2021.626616/full

Bispecific Antibodies: An Area of Research and Clinical Applications (Aug 2023)

FDA perspectives on bispecific Antibodies (BsAbs)

https://www.fda.gov/drugs/news-events-human-drugs/bispecific-antibodies-area-research-and-clinical-applications

1. **"BsAbs have joined mAbs on the therapeutic antibody stage**. …their synergistic (or cooperative) features may produce **more significant treatment effects"**.
2. "Right now, most BsAbs in development aim to treat **cancer**, but others are focused on **chronic inflammatory, autoimmune and neurodegenerative diseases; vascular, ocular, hematologic disorders and infections"**.
3. **"**In 2021, FDA finalized a **guidance on BsAb development programs**, which discusses aspects for Chemistry, Manufacturing and Controls (CMCs) as well as nonclinical and clinical development programs. …the guidance recommends types of data to support BsAb approvals."
   - https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bispecific-antibody-development-programs-guidance-industry
4. "FDA staff have been conducting **research to analyze different BsAb molecular formats using physiochemical and biological approaches**….. **to understand how different formats of BsAbs impact quality aspects** (e.g., product characterization, bioassay or potency assay development, and stability)."

Bi-specifics formats, BsAb (IgG-like)

Examples: Duobody (GenMab), CrossMab (Roche), Wuxibody (WuXi Biologics), XmAb (Xencor)

Pro's:

- Easier purification due to Fc region
- Typically, good stability and solubility seen
- Expected half-life good
- High affinity, and thereby good biological activity

Con's:

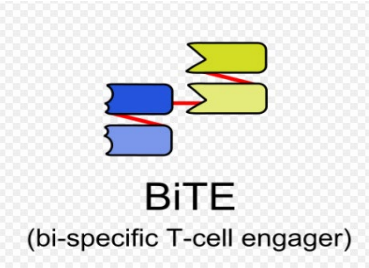
- Correct pairing of HC and LC not straight-forward
  - **lower yield**
- Principle enabling hetero-dimer form: Knobs-in-Holes technology
- Some methods/formats are patented; some can be in-licensed or used at CDMOs

Bi-specifics formats, non-IgG

Examples: BiTE (Amgen), DART (Macrogenics), TandAbs (Affimed)

Pro's

- One chain structure possible, ie no need for correct hetero-dimer pairing
- Very stable structure may be found
- Good stability and half-life possible, but strongly dependent of format
- Better tissue penetration due to smaller size

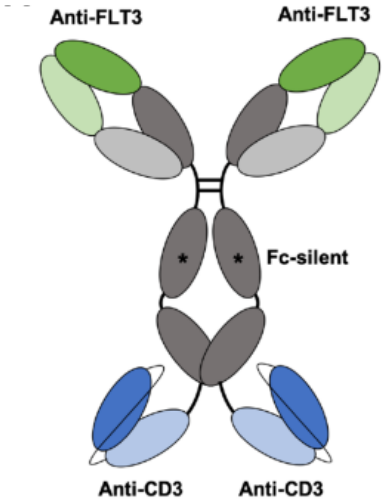


Con's

- Half-life shorter due to smaller size
- Non-natural format (risk of negative immune response)
- Many formats are patented –some can be in-licensed or provided at CDMOs
- Purification by affinity chromatography not straight-forward due to lack of IgG Fc region
  - **lower yield**

CLN-049 for Acute Myeloid Leukemia (AML) Treatment

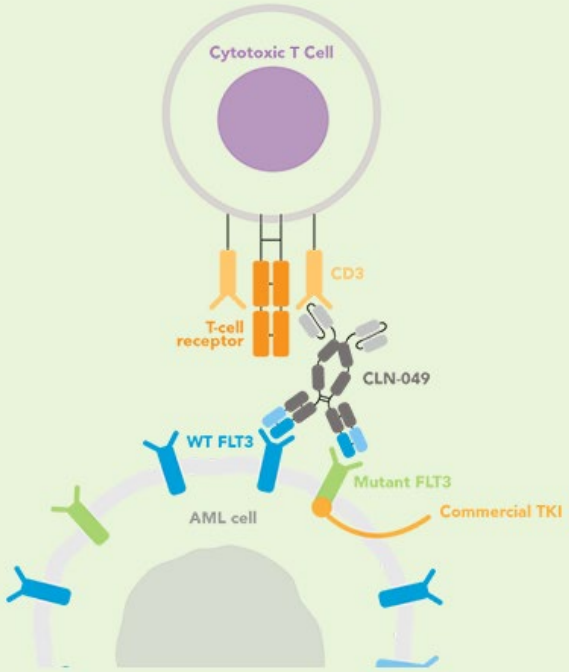
- AML is a rapidly growing cancer
- Patients have a 5-year survival rate of 10% or less in the relapsed setting
- With the goal to change the unmet need for treatment Cullinan Oncology developed a fully humanized bispecific homodimer molecule
- CLN-049 has specificity for FLT3 in its two Fab arms and specificity for CD3 in two single-chain variable (scFv) fragments fused to the heavy chain C-termini.
- The FLT3 surface receptor is expressed on AML cells in a majority of AML patients and constitutes a highly specific target antigen for immunotherapy
- T-cell engagers are characterized by low dose treatments, meaning that clinical material amounts typically are low
- An Fc-silent version was used as backbone for the molecule to prevent FcγR-mediated activation of T-cells

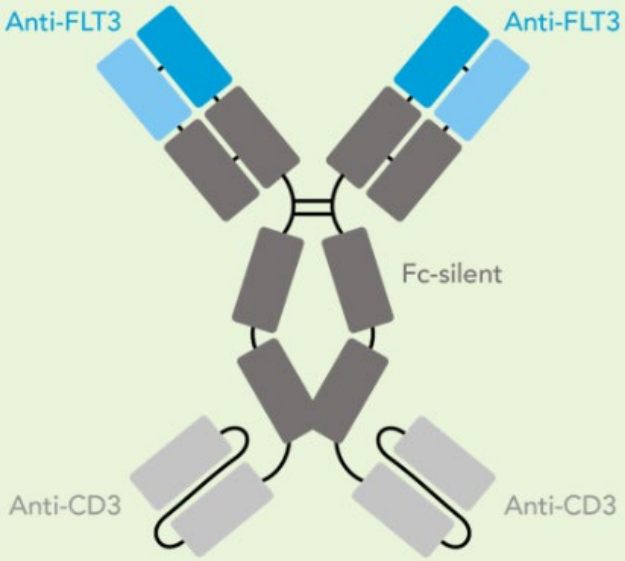


CLN-049 in the clinic

- A Phase 1, open-label, multicenter, first-in-human study of CLN-049 in patients with relapsed/refractory acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).

CLN-049 Mechanism-of-Action





Mechanism Of Action

Based on preclinical models, CLN-049 is a novel T cell engaging bispecific antibody candidate designed to simultaneously bind the extracellular domain of FLT3 on AML cells and a CD3 receptor on T cells. This interaction redirects the patient's effector T cells toward cancer cells and enables T cell-mediated cancer cell death. By binding the extracellular domain of FLT3, CLN-049 is capable of targeting AML cells independently of their FLT3 mutational status. CLN-049 was designed to have high affinity for FLT3 to also target AML cells with low overall levels of FLT3 expression. The silent Fc domain and CD3-targeting domains that are functionally monovalent aim to minimize non-specific T cell activation and reduce potential toxicity.

CLN-049 CMC Development

- A CLN-049 production clone was developed in CHO cells
- A manufacturing process at a titer of 3 g/L was used to produce CLN-049
- The Fc region of the molecule enabled capture by Protein A to high purity assessed by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion chromatography (SEC)
- Mass spectrometry confirmed the anticipated molecular weight of the heavy chain/scFv fusion
- CLN- 049 showed a high overall melting temperature of 77°C as determined by differentiating scanning calorimetry (DSC).
- Binding characteristics were assessed using Biacore and sandwich ELISA

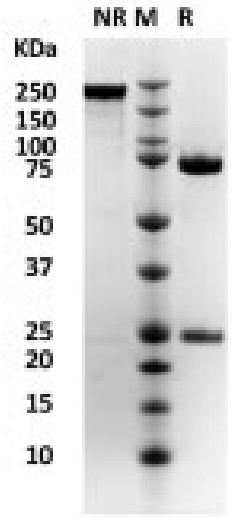
Mehta NK, et al. J Immunother Cancer 2022;10:e003882. doi:10.1136/jitc-2021-003882

The homodimer structure of CLN-049 most likely facilitated expression and correct folding of the molecule

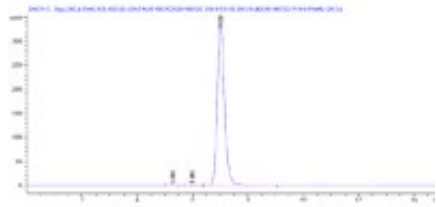
CLN-049 Structural and Functional Assessment

- The symmetric, IgG-based design should allow for characteristics typical of a monoclonal antibody process, such as production process, test methods, and stability
- Extended characterization is often needed to fully understand the properties of the bispecific

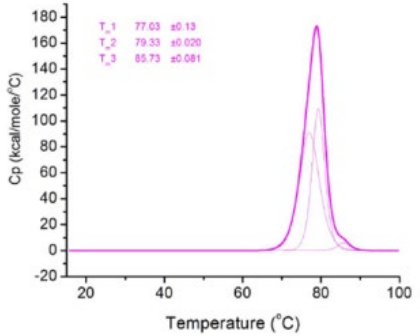
SDS-PAGE



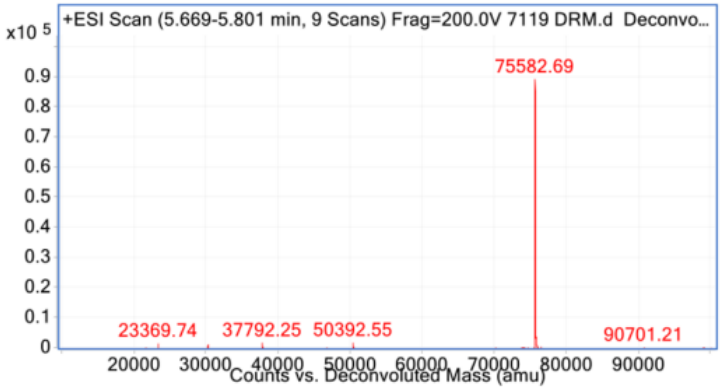
SEC



DSC



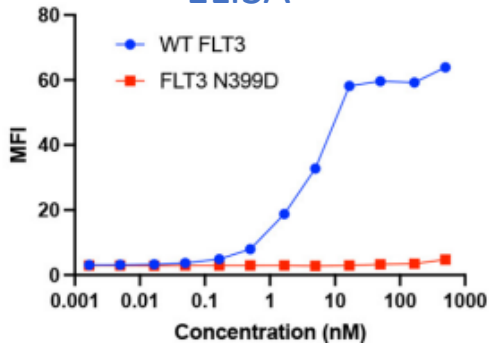
Mass Spectrometry



Biacore

Ligand	Ka ($M^{-1}s^{-1}$)	Kd (s^{-1})	K_D (nM)
FLT3 (Monovalent)	2.09E+05	3.80E-02	182
FLT3 (Avid)	9.74E+04	1.53E-03	15.7
PDGFRα (Avid)	ND	ND	>1E+06
PDGFRβ (Avid)	ND	ND	>1E+06
VEGFR2 (Avid)	ND	ND	>1E+06
VEGFR3 (Avid)	ND	ND	>1E+06

ELISA



Mehta NK, et al. J Immunother Cancer 2022;10:e003882. doi:10.1136/jitc-2021-003882

Bispecifics approach for Intravitreal Treatment of Diabetic Macular Edema

Integrin involvement in retinal diseases

- Current anti-VEGF treatment has not achieved maximal efficacy for long-term visual acuity gains
- I**ntegrins** play an important role as mediators of effector pathways leading to pathological disease states in the retina

Table 1. Role of Integrins in Retinal Diseases[c,d,e]

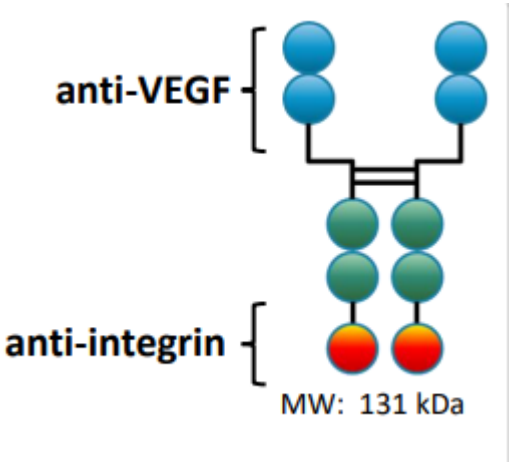
Pathophysiology of the Condition	Cellular and Molecular Mechanisms	Integrin(s) Involved	Related Retinal Diseases
Retinal/ Choroidal neovascularization	ECM remodeling, endothelial cell migration, proliferation, survival and adhesion, sprouting of vessels and tube formation	αvβ3, α5β1, αvβ5	DME, nAMD, DR, RVO, ROP
Retinal vascular permeability	Endothelial cell migration, proliferation, stability related to growth factor, VEGF mediated permeability and other integrin dependent mechanisms	αvβ3, α5β1, αvβ5	DME, nAMD, DR, RVO
Retinal inflammation	Immune cell recruitment, activation, migration, release of inflammatory factors and signaling	αvβ3, α5β1	DME, GA, PVR
Retinal fibrosis	Glial cell, astrocyte, microglial, and fibroblast proliferation, cell migration, and ECM remodeling	αvβ3, α5β1, αvβ1, αvβ5, αvβ6	nAMD, DR, ROP, PVR

CMC development of AG-73305

- Based on the growing knowledge of factors involved in Diabetic Macular Edema (DME) and other eye diseases, **Allgenesis** decided to develop a bispecific molecule targeting both VEGF and various integrins

- The molecule was termed AG-73305 and it is a genetically engineered homodimeric IgG1 Fc-fusion protein with binding properties for VEGF at the N-terminal and for integrins at the C-terminal

AG-73305



AG-73305 was designed to inhibit:

- **VEGF-A, VEGF-B and PIGFs**
- **αvb3, α5β1, αvβ1, αvβ5, αvβ6**

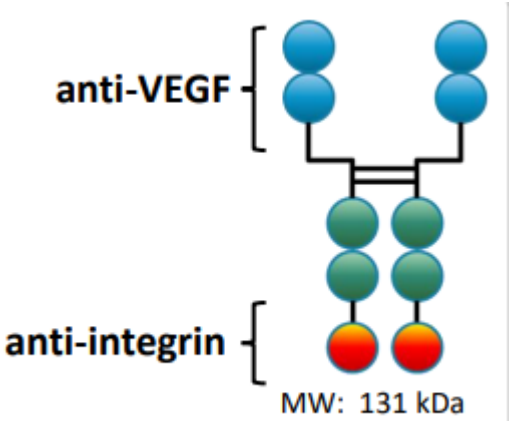
CMC Development of AG-73305

- The molecule could be contructed, produced and released in a manner very close to how monoclonal antibodies are produced
- The CMC development started with conventional synthetic codon-optimized gene synthesis and cloning into an expression vector suited for expression in CHO cells
- A fedbatch manufacturing process using chemically defined media and feeds was designed and developed.
- Standard protein A capture followed by chromatographic polishing steps were used in the downstream process as the bispecific contains protein A binding IgG constant domains.
- Virus inactivation orthogonal steps by low pH and nano-filtration were also shown to be feasible for this molecule
- The molecule was formulated at a relatively high final concentration to facilitate the eye injection

CMC Development Challenges

- Downstream yield was – as often seen for non-natural bispecifics – not as high as for the typical mAb;
- However, this was countered by the demonstrated efficacy of the molecule at a very low clinical dose and thus, sufficient amounts of early phase clinical material were easily produced in a 200 L disposable SOB.
- The release specification for AG-73305 resembles that of a typical mAb, except that activity assays for all functionalities of the molecule would be tested.
  - In this case, the early-stage release specification contains 3 ELISA binding assays.

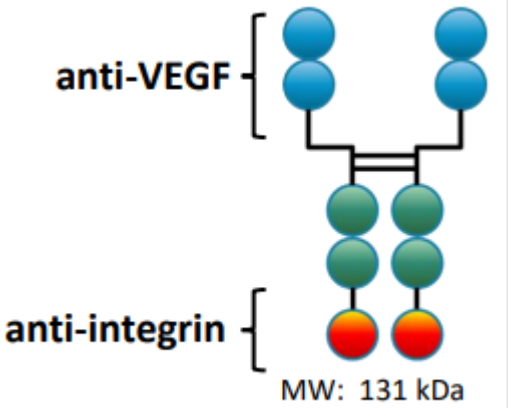
AG-73305



Clinical Development of AG-73305

- AG-73305 is formulated at 40 mg/mL as an ophthalmic solution and delivered by ocular intravitreal injection, ie directly in the eye
- Initial dose-range finding clinical studies showed that 0.5 and 1 mg/eye were well tolerated (single dose, 6 months follow-up)
- AG73305 showed dose-dependent improvements in best-corrected visual acuity (BCVA) and Central Subfield Thickness (CST) in non-naïve and naïve DME patients

AG-73305



AG-73305 in the clinic:

- Phase 2a study ongoing with AG-73305, repeat-dose comparator-controlled phase 2b study being planned

Nobel Prize Chemistry Laureates 2022

The Nobel Prize in Chemistry 2022

The 2022 chemistry laureates

The Nobel Prize in Chemistry 2022 was awarded to Carolyn R. Bertozzi, Morten Meldal and K. Barry Sharpless "for the development of click chemistry and bio-orthogonal chemistry".

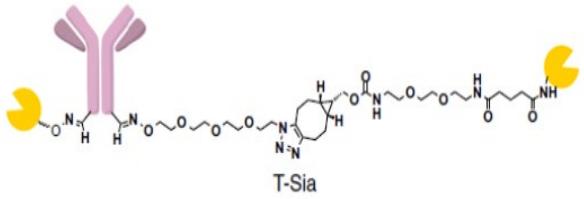
Sharpless and Meldal have laid the foundation for a functional form of chemistry – *click chemistry* – in which molecular building blocks snap together quickly and efficiently.

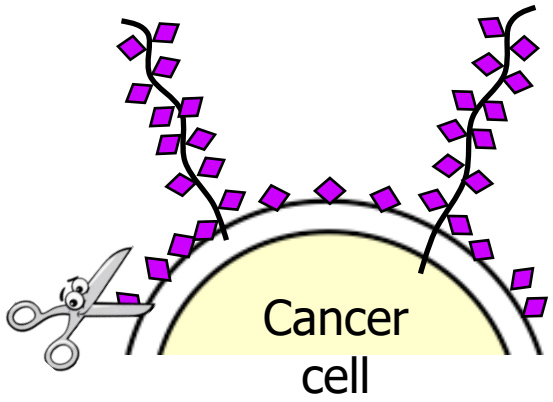
Carolyn Bertozzi has taken click chemistry to a new dimension and started utilizing it in living organisms.

The Origin of the Foundational Concept of *EAGLE*

Carolyn Bertozzi
Stanford University
Co-founder of Palleon

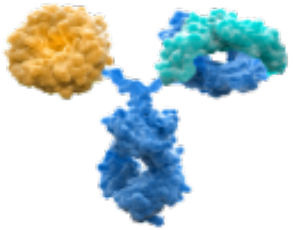
2022 Nobel Prize in Chemistry





Desialylation of cancer cells led to enhanced NK Cell killing of tumor cells

EAGLE



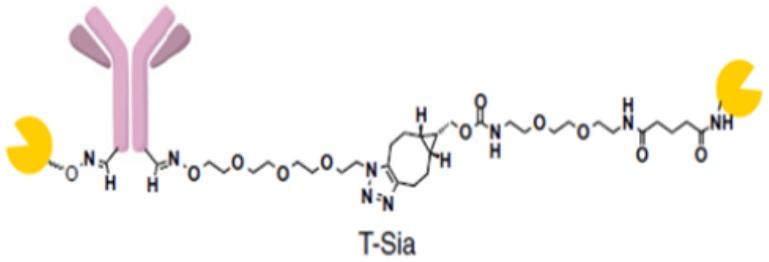
Therapeutic Platform

Enzyme-based drugs that target pathological glycans

Xiao H, Woods EC, Vukojicic P, Bertozzi CR PNAS. 2016

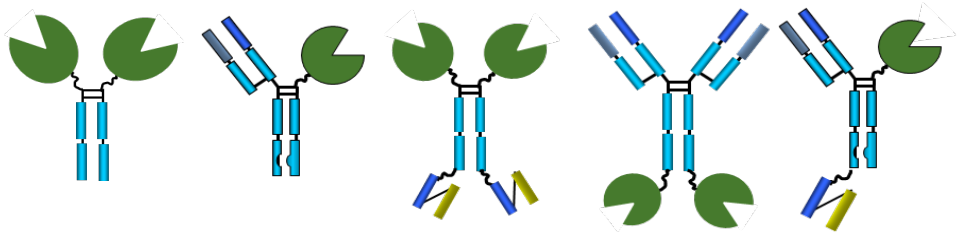
Engineering and Developing of *EAGLE* Therapeutic Platform

Initial Concept



- Bacterial sialidase (high immunogenicity risk)
- Chemical conjugates

EAGLE Therapeutic Platform



- Engineered human sialidase
- Genetic fusions

Engineering Human Sialidase Neu2 to Improve Developability

Human wildtype sialidases (Neu1-4) are not developable

5-Day HEK Culture (Shake Flask)	Extremely low expression yields (< 1 mg/L) ~90% aggregates after protein A purification Significant loss of enzyme activity after purification
14-Day CHO Fed-Batch Culture (Bioreactor)	Zero yield

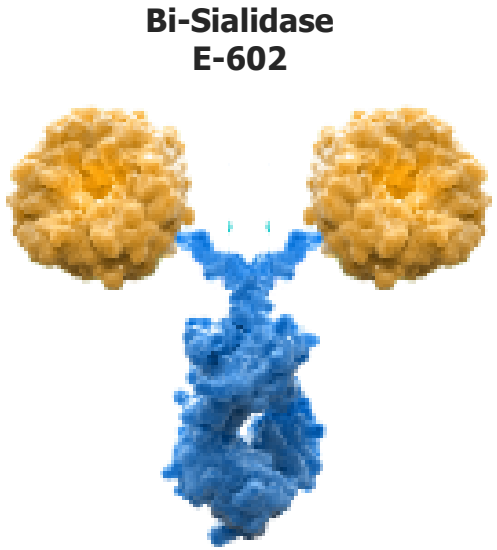
Engineered Human Sialidases have good developability profiles

	Titer (16-day Fed-Batch CHO Cell Culture)	***EAGLE #*** (Drug candidates)
Wild-type Neu2	0	N/A
eNeu2 v1	0.1 g/L	N/A
eNeu2 v2	2.8 g/L	E-602
eNeu2 v3	4.0 g/L	E-434

E-602 CMC and Clinical Development

CMC aspects

- E-602 is expressed in CHO cells by a conventional fedbatch process with a titer of ~3 g/L, followed by purification using protein A capture, chromatographic polishing steps and standard orthogonal virus clearance steps.
- The purification yield is – as often seen for bispecifics - relatively low compared to standard mAb yield. This may often be caused by increased levels of aggregates with the different format employed.
- For this particular molecule, removal of HCP turned out to be more of an issue than in a standard mAb process. The solution (so far) has been to add an additional chromatographic step.

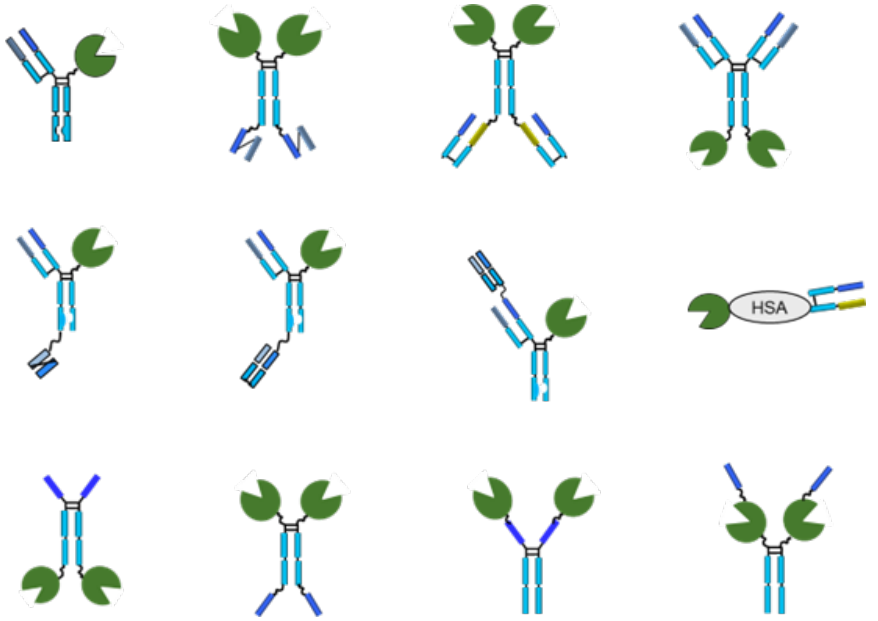


E-602 in the clinic

- Clinical development is done as a monotherapy with the potential of combinations with T-Cell Engagers, ADCC, T-Cell checkpoints, cell therapy, and chemotherapy agents.
- Human safety and proof of mechanism has been established and the molecule is ready for Phase II

Next step: Bi-Functional Targeted Sialidases

- The targeting arm enables **more efficient desialylation of antigen-expressing cells** than Bi-Sialidase (E-602)
- **Multiple configurations**
- The targeting arm introduces **additional MoAs**
  - ADCC
  - Signal transduction inhibition
  - Other immune-modulatory mechanisms



Immune Cell and Tumor Cell Targeted	Immune Cell Targeted	Tumor-Associated Antigen (TAA)-Targeted			
PD-L1-Sialidase	**PD1-Sialidase**	**HER2-Sialidase**	**TROP2-Sialidase**	**Nectin4-Sialidase**	**Other TAA-Sialidases (B7H3, etc.)**

Summary: Bispecifics Status and Perspectives

- Development and market approval of bispecifics is growing fast – due to scientific creativity envisioning novel treatment options
- T-cell engagers have been the fastest bispecific formats to obtain market approval
- CMC development, product characterization and release has proven to be relatively easy (but most often with lower yields compared to mAbs) – for carefully selected formats!
- Release specification follows ”standard” specifications with some exceptions, for example the number of binding/functional assays
- Overall, process development typically need more attention compared to mAb processes, to potentially deal with:

  - Aggregates
  - In-correct pairing of multichain formats
  - Stability issues, eg to low pH
  - Half-life (especially for non-BsAbs)

  } Lower yield
  Heterodimers are more challenging

Acknowledgements

BioProcess Technology Group at BDO

- Dawn Ecker, Patti Seymour
- **CLN-049** (phase 1) developed by Cullinan Oncology, Cambridge, USA (www. cullinanoncology.com)
- **AG-73305** (phase 2) developed by Allgenesis Biotherapeutics, Taipei, Taiwan (www.allgenesis.com)
- **E-602** (phase 1) developed by Palleon Pharmaceuticals, Waltham, USA (www.palleonpharma.com)

Creating new standards of care for patients

Treatment of ophthological (eye) diseases

Unlocking the Therapeutic Potential of Glyco-Immunology

Frederiksborg slot/Hillerød Castle, Denmark