Bioassays Throughout Drug Development and the Product Life Cycle

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Bioassays
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What is Pharmaceutical Quality?

- A quality product of any kind consistently meets the expectations of the user
  - Drugs are no different
- Patients expect safe and effective medicine with every dose they take
- Pharmaceutical quality is assuring every dose is safe and effective, free of contamination and defects
  - It is what gives patients confidence in their next dose of medicine

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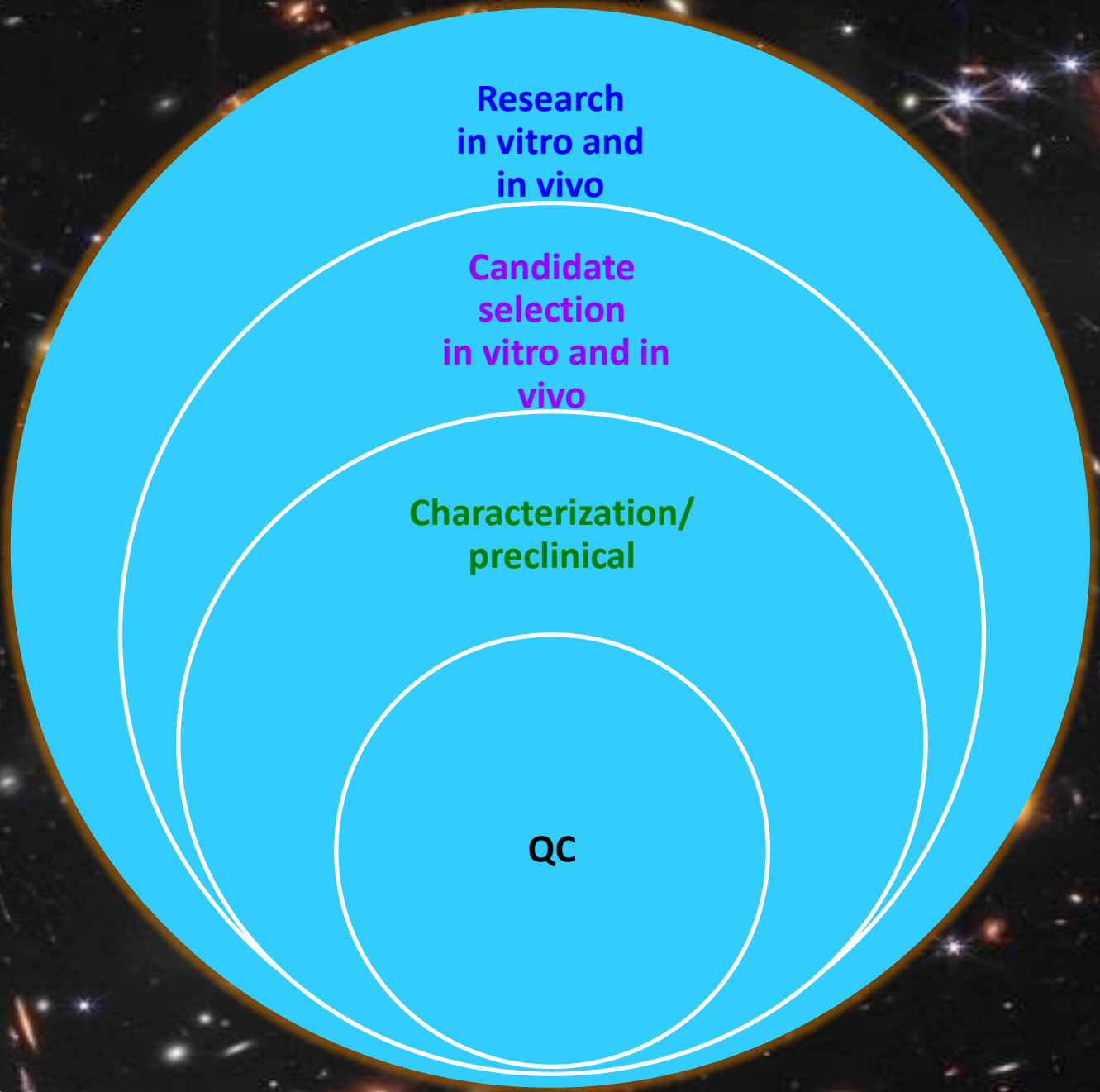
Disclaimer

- The views presented today do not represent official FDA policy, but rather represent my opinion based on my experience as a product quality reviewer of in the Office of Biotechnology Products/FDA.

Objectives

- Universe of bioassays
- Drug development and candidate selection
- Lessons learned turned into next generation products
- Challenges in designing bioassays and potency assays for ADCs and BsAbs
- Common assays across product classes
- New bioassays for old products
- Are bioassays always needed for QC purposes?
- Take home messages

Universe of Bioassays



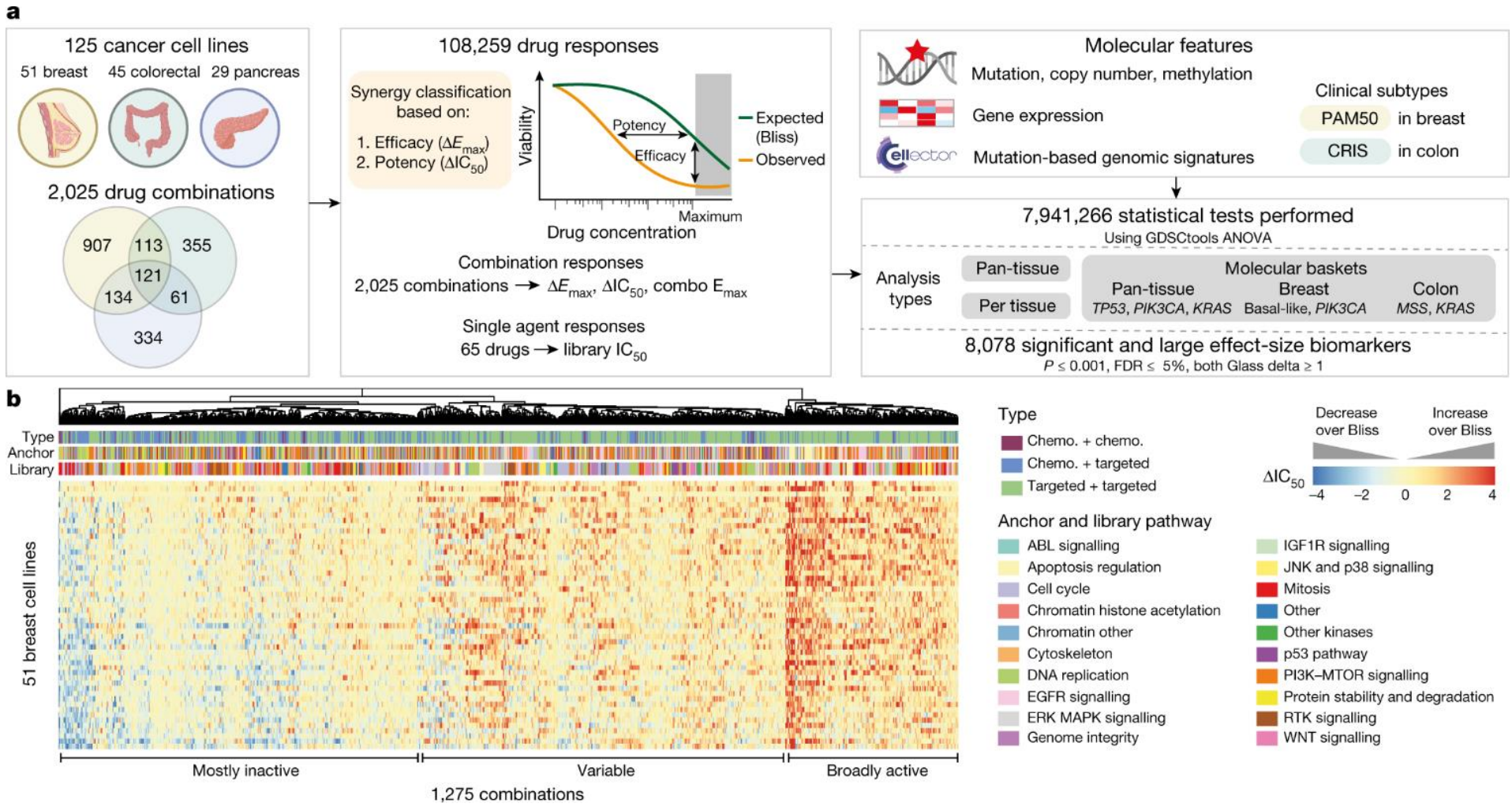
Galaxy cluster SMACS 0723 as seen by Webb

Drug development failures

- Technological advances have produced … data on the biological cause of disease, moving these discoveries into treatments has been far more difficult
- The high failure rate in drug development is largely the result of poor target selection and preclinical experiments in cell-based or animal models that do not properly represent human disease.
- Accelerating Medicines Partnership (NIH, FDA, EMA, industry and non-profit organizations) aims to improve understanding of therapeutically relevant biological pathways (AD, autoimmune and immune mediate diseases, RA/lupus, bespoke gene therapy consortium, common metabolic diseases, Parkinson's, and schizophrenia)

Accelerating Medicines Partnership https://www.nih.gov/research-training/accelerating-medicines-partnership-amp

Multidisciplinary, big science in the 3rd decade of the 21st century

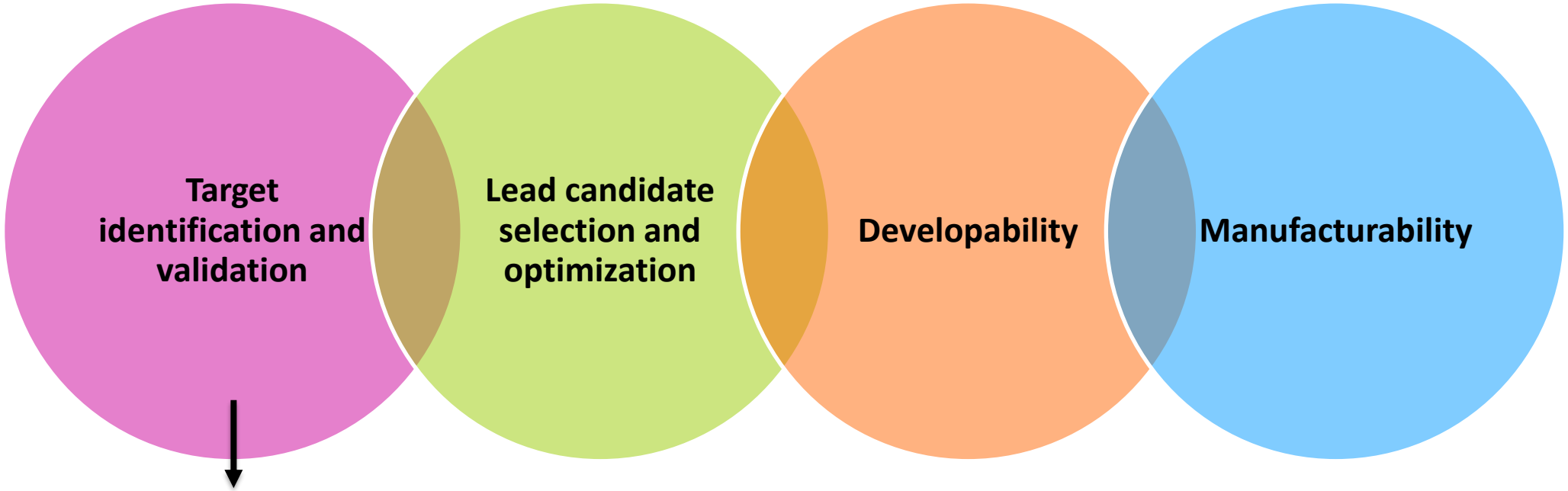


Drug combination screen heat map of ΔIC50 values in breast cancer cell lines

- 2,025 pairwise drug combinations in 125 breast, colorectal and pancreatic cancer cell lines
- Found combinations of drugs with synergistic effects in specific cell lines.
- Overall synergy was rare, but 192 drug combination-tissue pairs were synergistic in at least 20% of cell lines in a tissue.

Effective drug combinations in breast, colon and pancreatic cancer cells Jaaks et al. Nature 2022 https://www.nature.com/articles/s41586-022-04437-2

Early drug development activities



- Review of literature
- In-house research
- Causal role in disease and is the target druggable?
  - Known 3D conformation?
- Available animal models or need to generate a surrogate mAb?
- Organ-on-a-chip/3-D spheroids?
- Identify potential biomarkers
- Develop Target Product Profile

- There is overlap among these early R&D activities, which require multi-disciplinary teams
- It helps to define the Target Product Profile at the beginning of the project

Role of bioassays in R&D activities to help define TPP, QTPP and make go/no go decisions to initiate clinical studies

Lead Discovery

- Target assessment
  - Evidence supporting rationale for selecting target
  - Experimental validation of target
- Screening preparation
  - Need reagents for assay development and screening, expression constructs, purified protein, etc.
    - Could take a long time to develop appropriate reagents and methods
- Lead optimization and characterization
  - Engineering for specific purpose (enhance or reduce specific functions)
  - Pharmacokinetic and immunogenicity de-risking strategies
  - Do you have the right assays to assess these features of the drug candidates?

Drug candidate selection challenges – avoiding pitfalls

- Drug candidate selection failures occur when the characteristics required for successful product development, especially before expensive studies are undertaken, are not considered early:
  - Product profile (related substances, etc.)
  - Stability
  - PK and bioavailability
  - Immunogenicity
- Biological potency is the most widely used selection test but needs to rely on the development of a well characterized assay based on the mechanism of action.
  - Binding assays are important, especially if affinity matters, but are not sufficient
- Effort up front at the selection stage can pay dividends later in development
  - Identify assays for product characterization, release and stability

Drug candidate selection challenges – avoiding pitfalls

- Gains in understanding a disease improves a target-based approach to lead discovery and candidate selection, but…
- “Targets engineered into simplified cell-based assays do not always behave the same as in the complex environment of intact organisms, and even results from gene engineering in model species may not translate to patients.”
  - GE Croston. 2017. The utility of target-based discovery. Expert Opinion in Drug Discovery 12:5 p 427-429

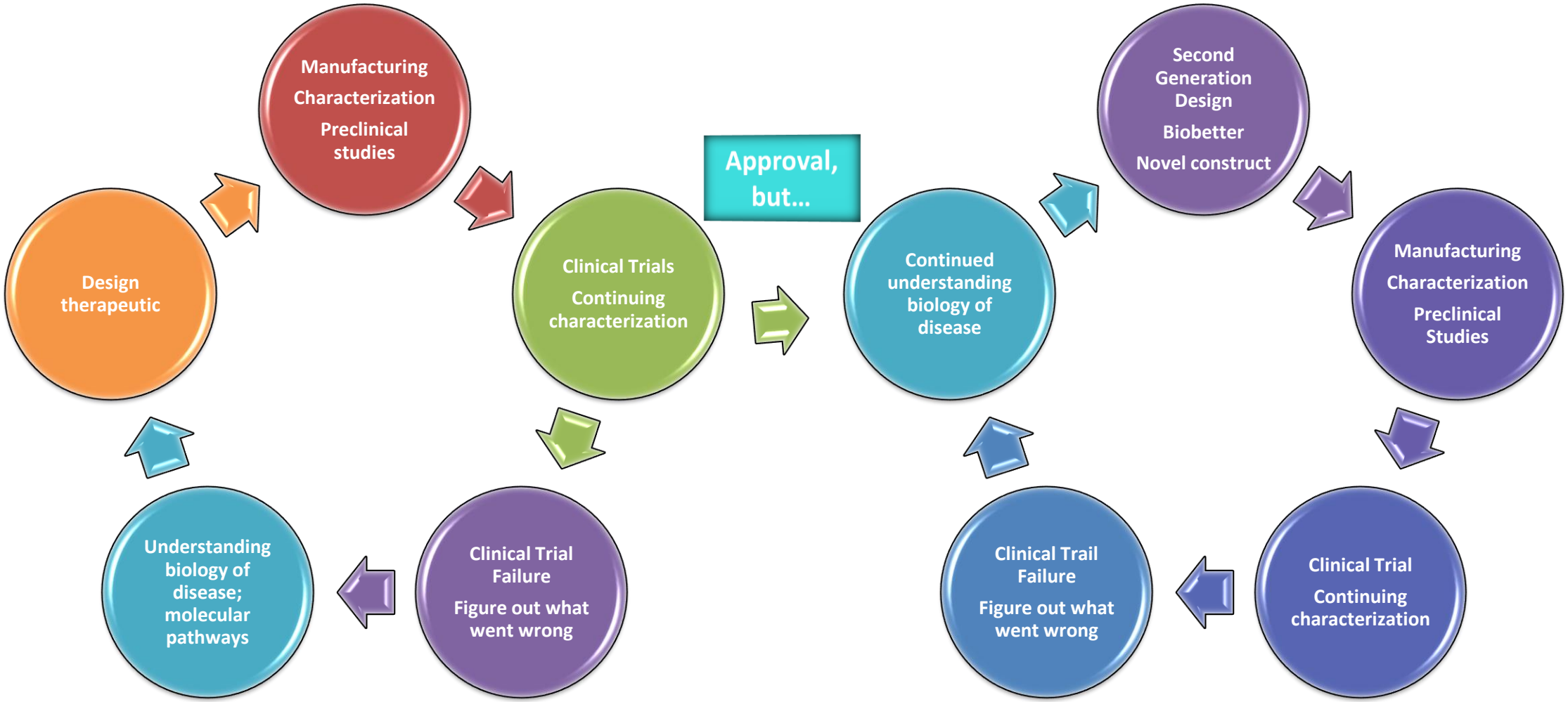
Drug candidate selection challenges – avoiding pitfalls

- **Problem:** Screening assay based on binding to a trimeric ligand. Assay conditions caused dissociation and lack of epitope expression. Lack of assay suitability testing.
- **Result:** Many excellent candidates lost.
- **Lesson learned:** Better understanding of underlying mechanisms of disease can help identify appropriate targets, but if the bioassays used to identify and select lead candidates are inadequate, it can result in failure later in clinical development.

Lessons Learned Lead to Next Generation Products

Carina Nebula as seen by Webb

Drug Development cycle: Continuous learning leads to new and better products



Next Generation Rational Design Products

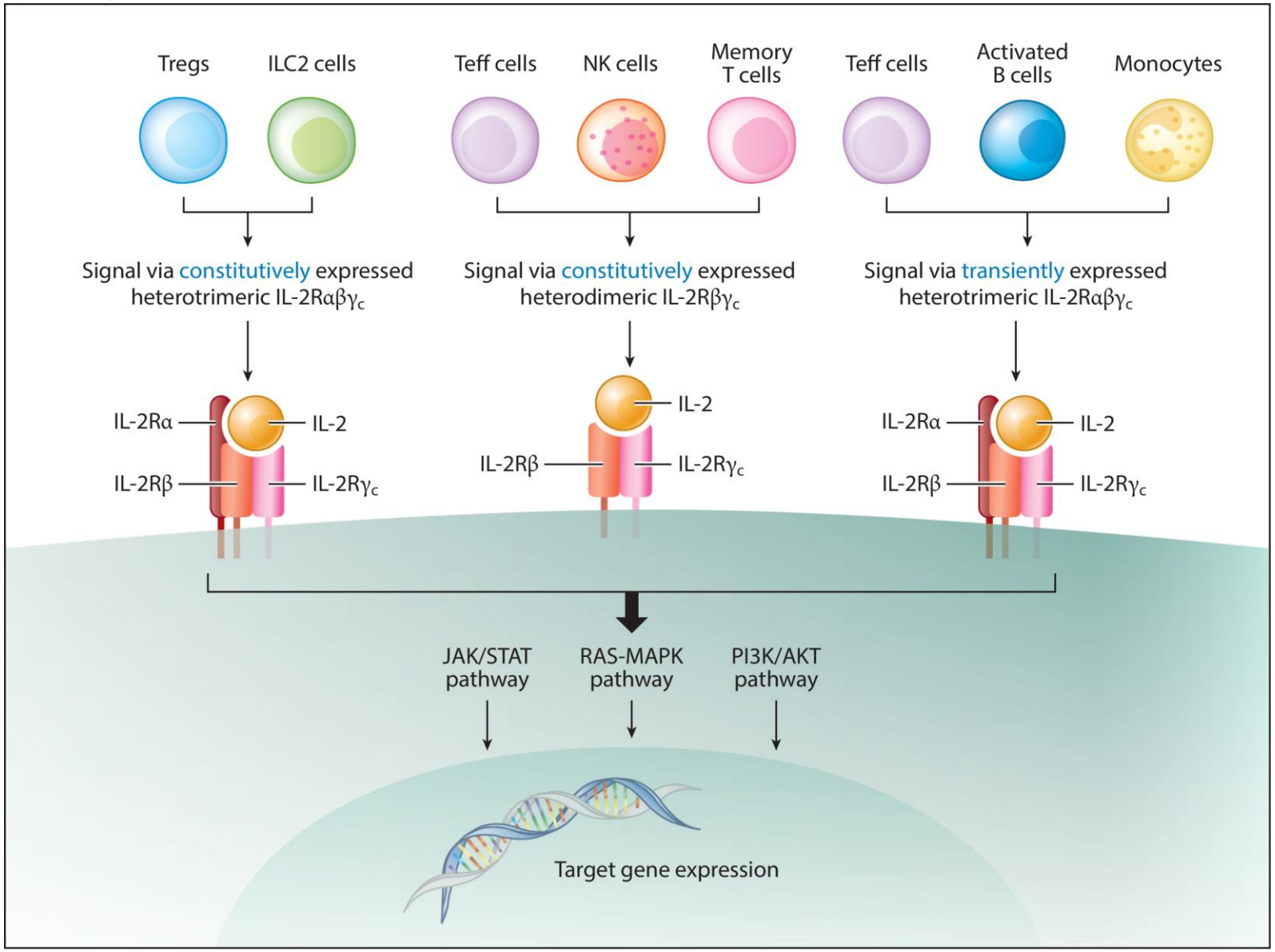
- Aldesleukin (IL-2) approved in 1992 for metastatic renal cell cancer and in 1998 for metastatic melanoma
- Uses a high dose
- Black box warning for capillary leak syndrome
  - may be associated with cardiac arrhythmias, angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.
  - “Proleukin administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.”
- Therapeutic value limited by frequent dosing (5 consecutive days), short half-life, severe toxicities and lack of efficacy in most patients
  - Lowering the dose to mitigate toxicity results in reduced responses.

IL-2 rational design

- Since 1992, have a better understanding of IL-2 activity and signaling pathways
- IL-2 has both immunostimulatory and immunosuppressive activity
  - Immunostimulatory activity in oncology indications
  - Immunosuppressive activity could be useful in autoimmune indications
- Opposite targets
  - Stimulate effector T cells in oncology and induce regulatory T cells for autoimmune indications, and not target either Tregs or Teff, respectively
- Re-engineering IL-2 for longer half-life, specific IL-2 receptor targeting for specific T cell subsets, and localization to specific tissues to optimize efficacy and reduce toxicity

IL-2 Signaling Pathways

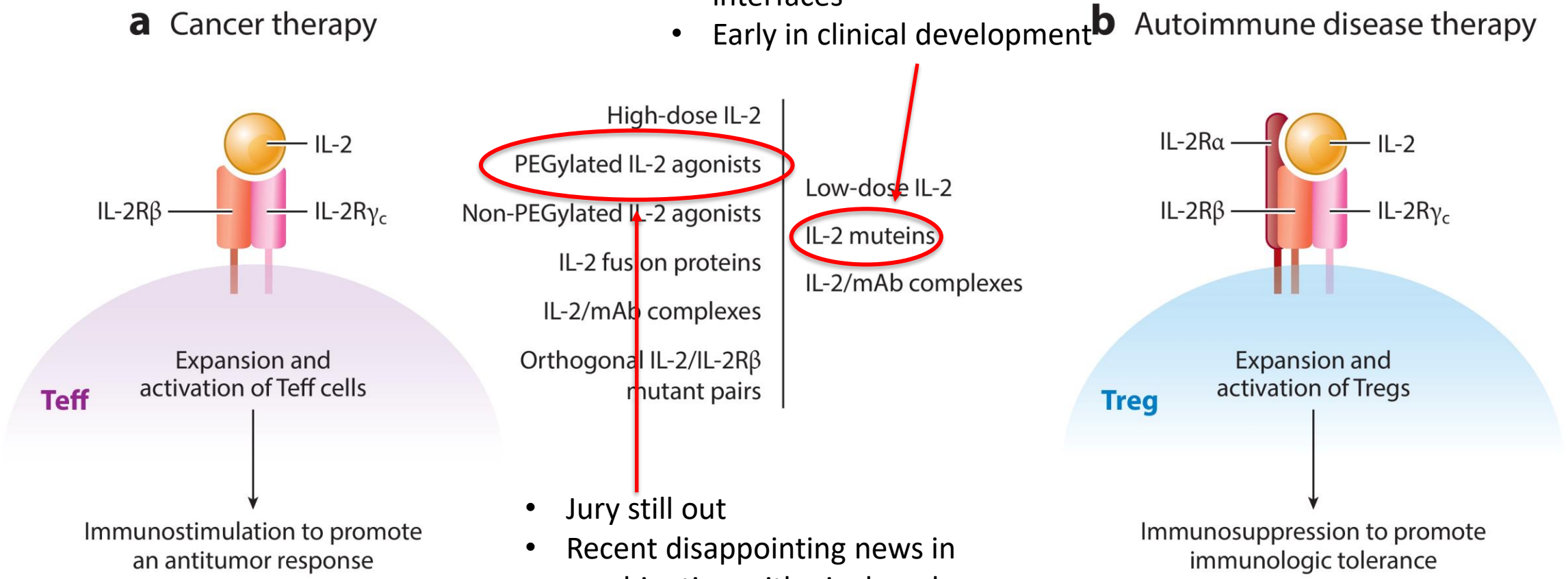
Signaling mediated by different IL-2R configurations expressed on different cell types



Overwijk et al. 2021. Engineering IL-2 to Give New Life to T Cell Immunotherapy | Annual Review of Medicine (annualreviews.org)

IL-2 Signaling Pathways

- Mutated to attenuate binding to IL-2R at different interfaces
- Early in clinical development



- Jury still out
- Recent disappointing news in combination with nivolumab
- Did not show clinical benefit

- **Exploit different IL-2R configurations to design IL2 molecules for different indications**
- **Need appropriate bioassays for intended activity**

[Overwijk et al. 2021. Engineering IL-2 to Give New Life to T Cell Immunotherapy | Annual Review of Medicine (annualreviews.org)]

Tragic failure – TGN1412

- IgG4 anti-CD28 superagonist to activate Tregs without co-stimulation through the TCR by an antigen presenting cell
  - Binds different epitope than more conventional anti-CD28 mAbs
- Intended to treat autoimmune diseases (activate Tregs to dampen disease) and B cell leukemia (expand T cells which are typically low)
- FIH in healthy volunteers
  - Dose was 500 times lower than used in animal studies
  - 10-minute intervals between dosing 6 volunteers
- All experienced severe cytokine release syndrome
  - Multiple organ failure
  - One volunteer lost toes and most fingers

Hunig, T. FEBS J. 2016 and Brown, KE Diseases 2018

TGN1412 – what went wrong?

- Preclinical studies in cynomolgus and rhesus monkeys
- 100% homology between human and cynomolgus and rhesus CD28 extracellular domain
- Repeat dose pre-clinicals study showed
  - Expansion of T cells with no signs of toxicity across doses
  - Additional tox study showed moderate increase in IL2, IL-5 and IL-6, but no clinical manifestations of CRS
  - **No signals from in vitro studies on human PBMCs**

- CD28 also expressed on tissue-resident CD4 T_{EM} in humans, but is down regulated on these cells in cynomolgus monkeys
  - T_{EM}s secrete proinflammatory cytokines
  - Possible role for FcγRIIb, even though TGN1412 is an IgG4 mAb
  - Didn't understand all the target cells recognized by TGN1412

Hunig, T. FEBS J. 2016 and Brown, KE Diseases 2018

Lessons learned and TGN1412 today

- Changed many aspects of clinical trial design with impact across products
  - Different approach to determine safe starting dose
  - Lengthened dosing intervals between patients and more diligent monitoring of patients
  - **Different designs for in vitro cell-based studies to detect cytokine release**

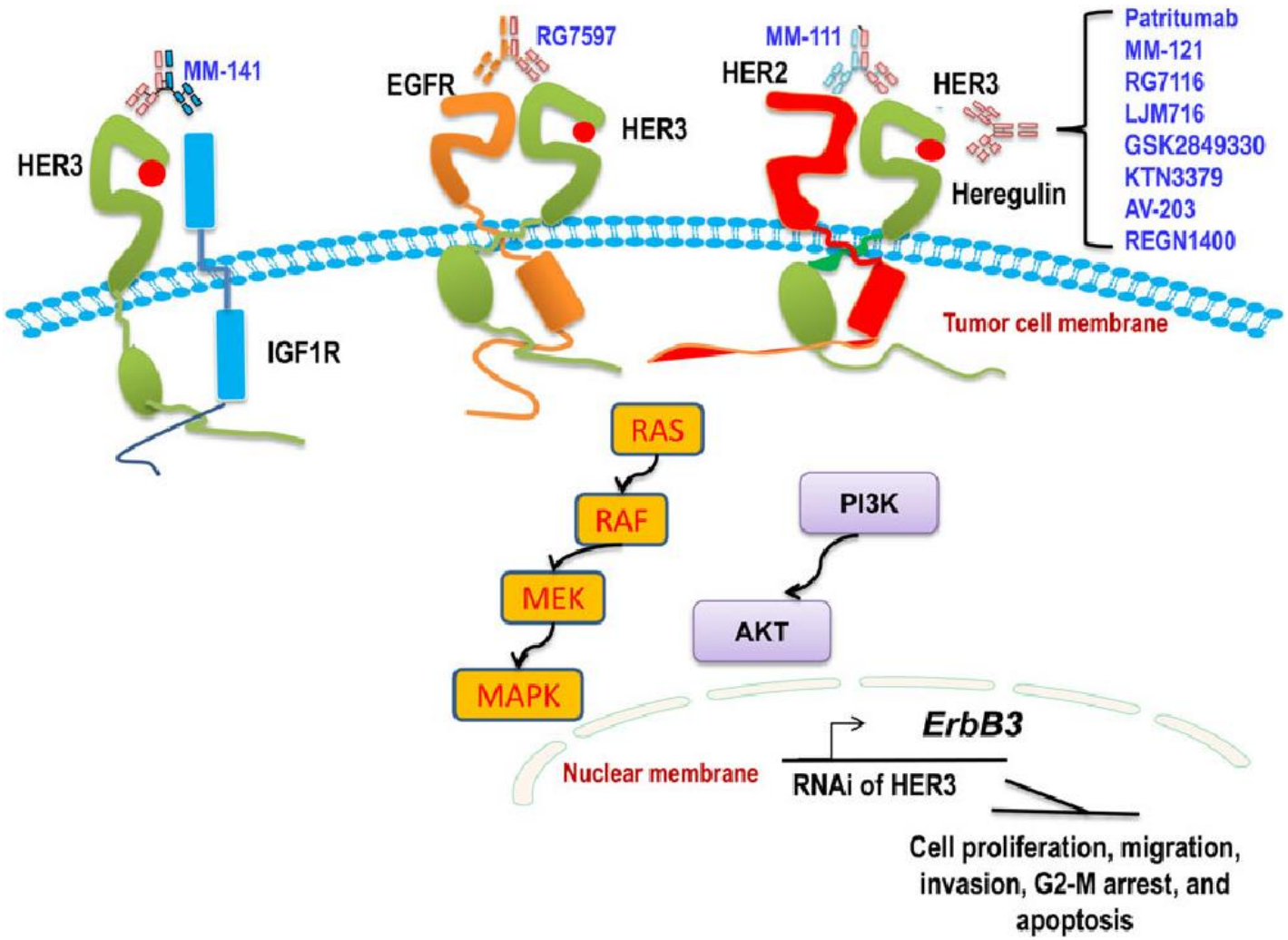
- TGN1412 is back in the clinic as TAB08 using a lower dose (1000- fold lower than initial clinical trial) that can still achieve Treg activation without release of proinflammatory cytokines
  - Healthy volunteer study completed
  - Clinical trials ongoing in RA, SLE, solid tumors

Hunig, T. FEBS J. 2016 and Brown, KE Diseases 2018

Other challenges when choosing candidate molecules

- TNF and TNFR family trimers
  - Do you need to engage all three molecules in the trimer or is one sufficient?
  - Soluble or membrane bound? If both, does binding soluble antigen interfere with the proposed MOA?
- Spike protein trimers, up and down configuration
  - Conformational changes
  - Target RBD, NTD and/or S2
  - Effector function or not?
- If a soluble ligand, does it have multiple receptors?
  - Is receptor expression limited on different cell types?
  - Are you targeting a specific receptor on a specific cell type?
- Homodimer vs heterodimer targeting, e.g., HER/EGFR family
  - How to best target dimers
- Epitope matters
  - Distance from membrane can affect antibody effector function activity
  - Agonist vs antagonist activity

Targeting HER3



Challenges targeting HER3

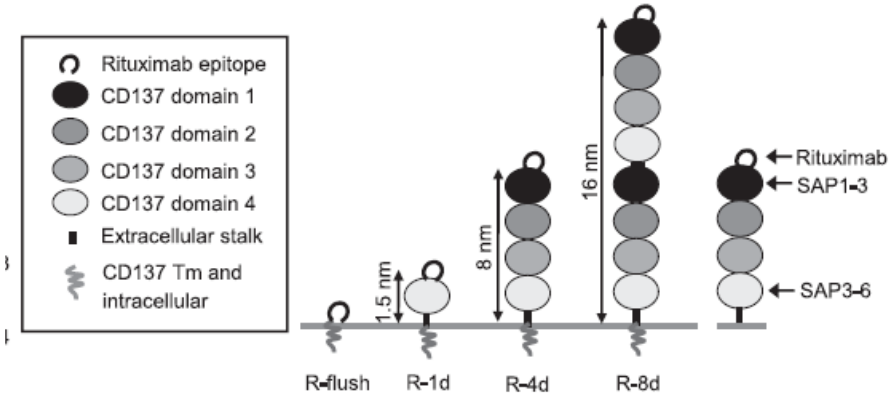
- Pleiotropic effects on downstream pathways
  - Different dimerization partners
- Lack of predictive biomarkers
- 1st gen mAbs largely unsuccessful
- Ongoing clinical trials
  - Next gen mAbs
    - Bind specific epitope at HER3 dimerization interface
    - Use in NRG1 fusion-positive cancers (biomarker)
  - ADCs
    - Use HER3 as tumor ag, don't need to block signaling
  - BsAbs
    - Generally, target HER3 and another member of the family
    - Adds inhibition of a second pathway

Zhang et al. Acta Biochim Biophys Sin, 2016, 48(1), 39–48

Epitope matters: distance from the cell membrane

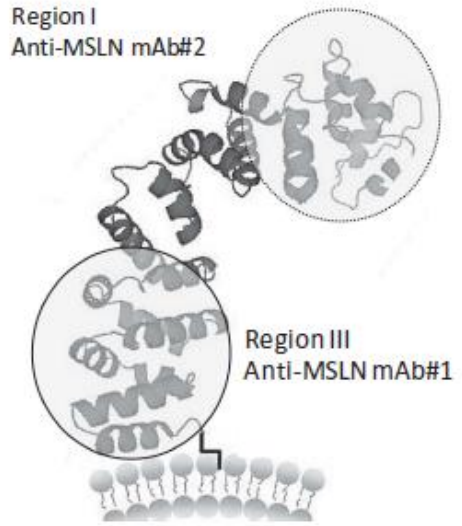
- Engineered rituximab or alemtuzumab epitopes on a CD137 scaffold of 1, 4 or 8 domains.
- ADCC and CDC activity favored a membrane proximal epitope while ADCP favored a more distal epitope

Cleary et al. JI 2017 Antibody Distance from the Cell Membrane Regulates Antibody Effector Mechanisms



- Similar finding for anti- mesothelin mAbs – better ADCC with membrane proximal mAb while the more distal targeting mAb had better ADCP activity

- Density of the target on the cell and flexibility of the ab elbow region



Hatterer et al. mAbs 2020 Targeting a membrane-proximal epitope on mesothelin increases the tumoricidal activity of a bispecific antibody blocking CD47 on mesothelin-positive tumors

Epitope matters: linear or conformational, angle

Type I antibodies	Type II antibodies
Class I epitope	Class II epitope
Localize CD20 to lipid rafts	Do not localize CD20 to lipid rafts
High CDC	Low CDC
ADCC activity	ADCC activity
Full binding capacity	Half binding capacity
Weak homotypic aggregation	Homotypic aggregation
Cell death induction	Stronger cell death induction
Rituximab, ocrelizumab (2H7), ofatumumab (2F2)	GA101, tositumomab (B1)

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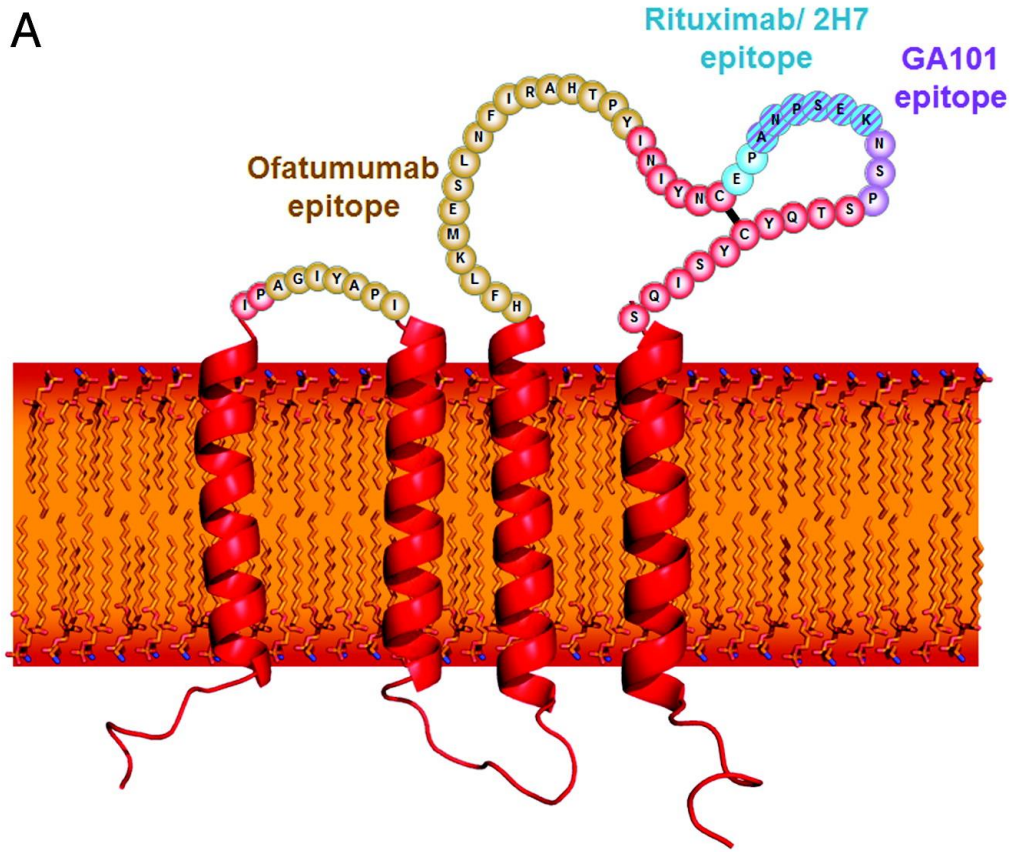
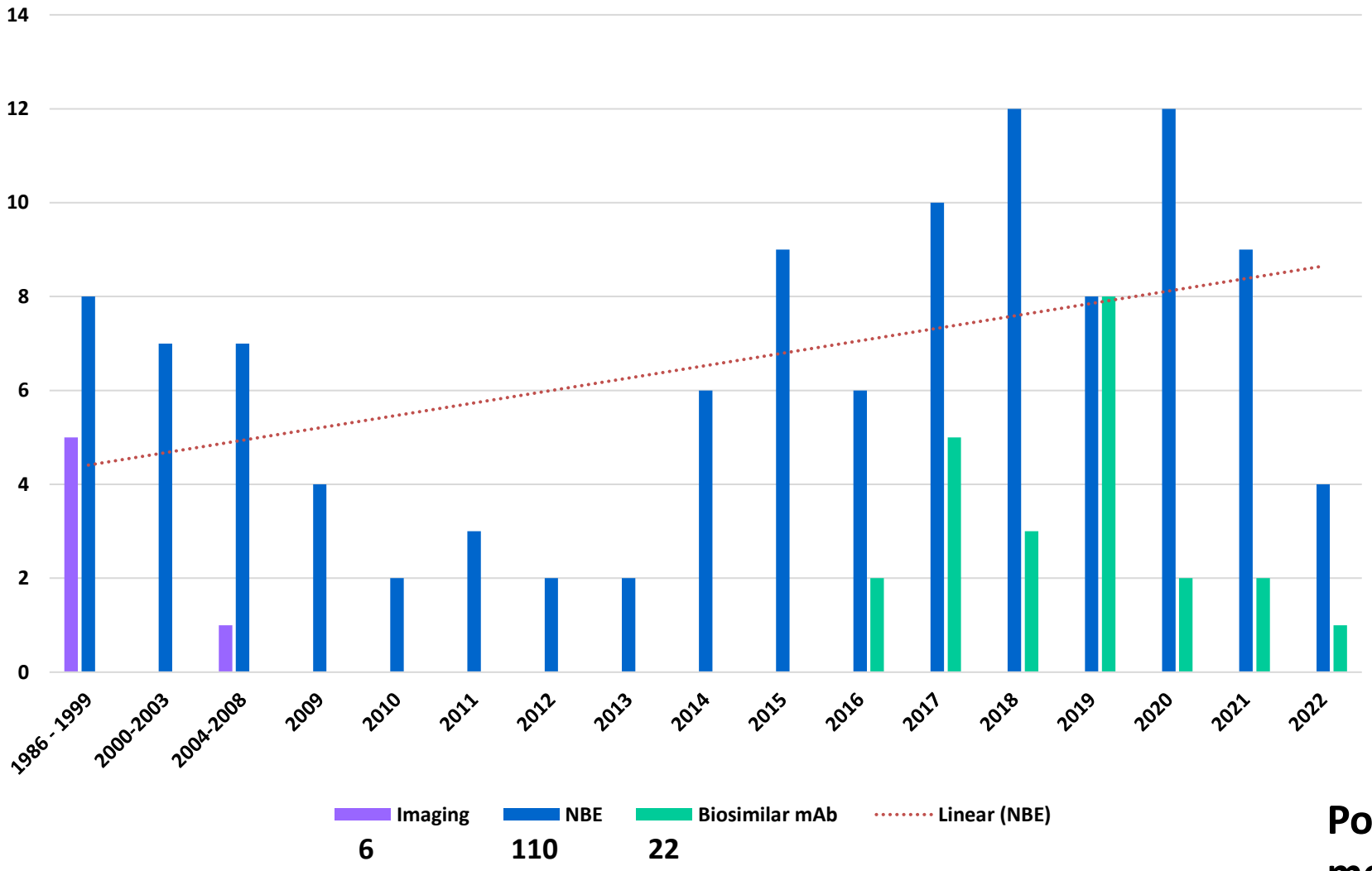


- The Type 2 mAb GA101 (obinutuzumab) has weak to no CDC activity.
- Different angles for binding CD20 between rituximab and obinutuzumab results in the differences for lipid raft localization, homotypic aggregations and cell death induction
- Ofatumumab is a Type 1 but its epitope spans the small and large loops. It has better CDC activity than rituximab

Klein, C. et al. mAbs, 2013, 5(1), 22–33


Planet Potency

mAb product approvals

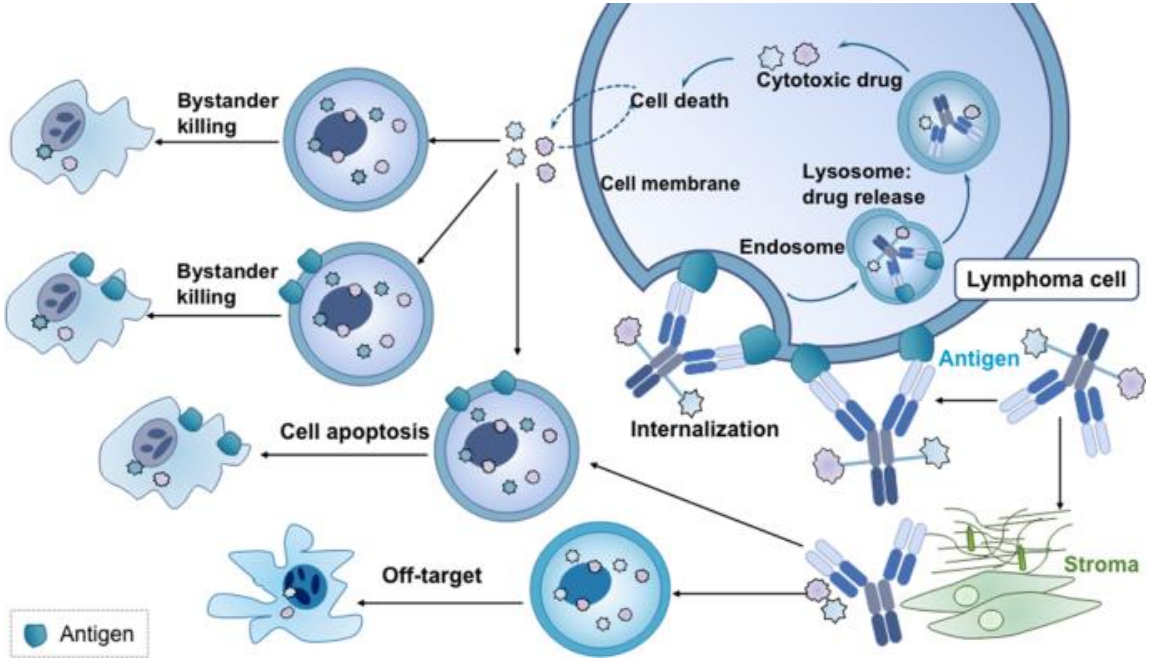


- 7 glycoengineered
- Numerous Fc-engineered
- 11 ADCs
  - 3 different DNA damaging agents
  - 2 different microtubule inhibitors
  - 1 protein synthesis inhibitor
- 5 BsAbs
  - 1 TCE with no Fc
  - 3 full length mAb structures
  - 1 TCR construct
- 2 cocktails
- 11 Fc-fusion proteins
  - 7 receptor
  - 2 peptide
  - 2 coagulation factor
- 1 Fc region

Potency assays and characterization methods should reflect the intentional design of the molecule

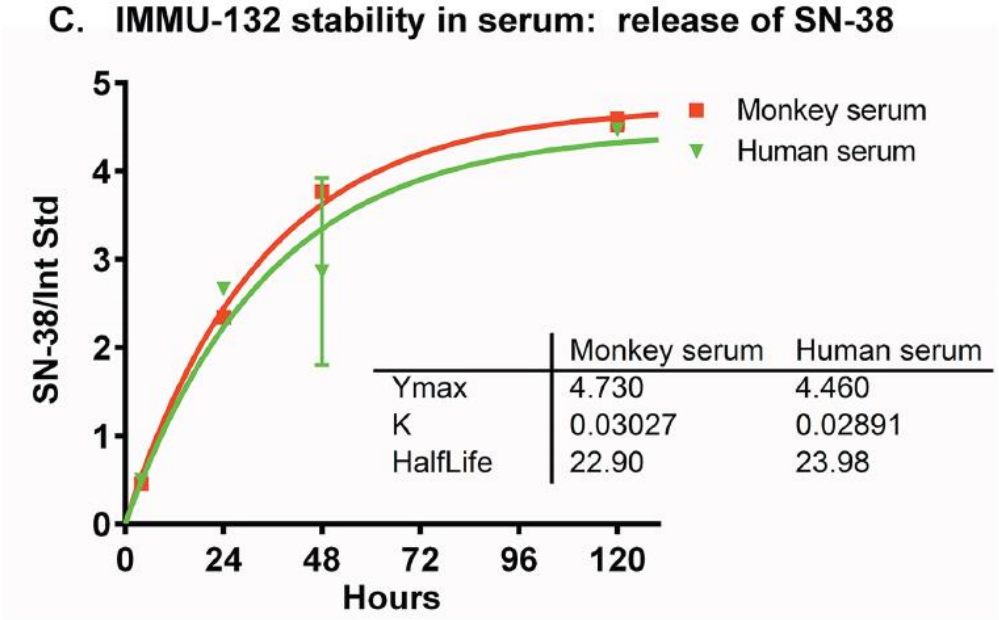
ADC mechanisms of action

- Bind target
- Internalize
- Release payload
- Disrupt internal target (tubulin or DNA)



Chu et al. 2021 J Hem and Onc
https://jhoonline.biomedcentral.com/track/pdf/10.1186/s13045-021-01097-z.pdf

- In addition to internalization, some linkers (sacituzumab govitecan) designed to release some payload prior to internalization for bystander effects.



In serum at 37°C,SN-38 is released from the conjugate with a half-life of ~1 day. Goldenberg et al. 2015 Oncotarget 6:26 p22496

ADC challenges for bioassays and potency assays

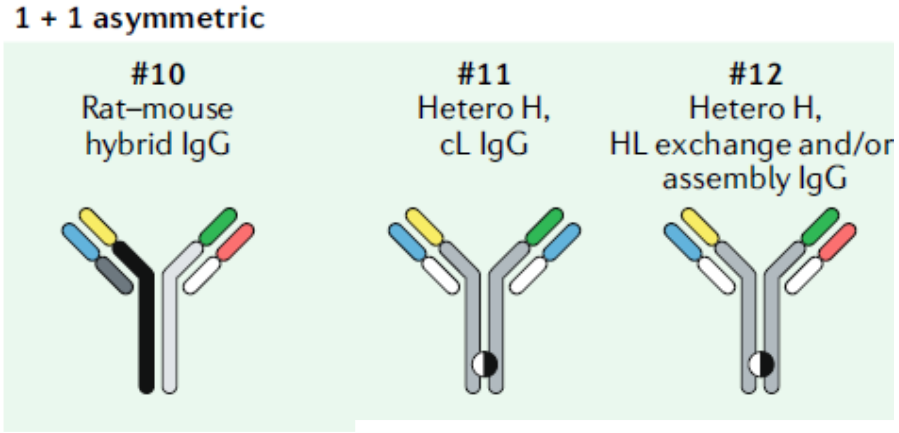
- Need to characterize the mechanism(s) of action as appropriate for the specific payload and mAb
  - Binding (mAb intermediate, DS, DP)
  - Cytotoxicity (DS and DP) (indicates binding, internalization, release of payload and cell killing)
    - Negative control (cell line that doesn't express target) to demonstrate specificity
  - mAb effector functions (mAb, DS and DP?)
- When payload intentionally released prior to internalization for bystander effects…
  - This complicates the cytotoxicity assay
  - Use cell line that doesn't express target to assess bystander effects?
- Need a justification for assays that should be used for release and stability assays
  - All assays used across mAb intermediate, DS and DP may not be needed for each by the time of a BLA.
  - May not need binding assay for DS and DP once it is shown that conjugation does not affect binding, unless binding assay is a better stability indicating method than cytotoxicity assay.
  - Drug to antibody ratio (DAR) useful as a control for potency and can help ensure consistent dosing and safety (off-target effects)

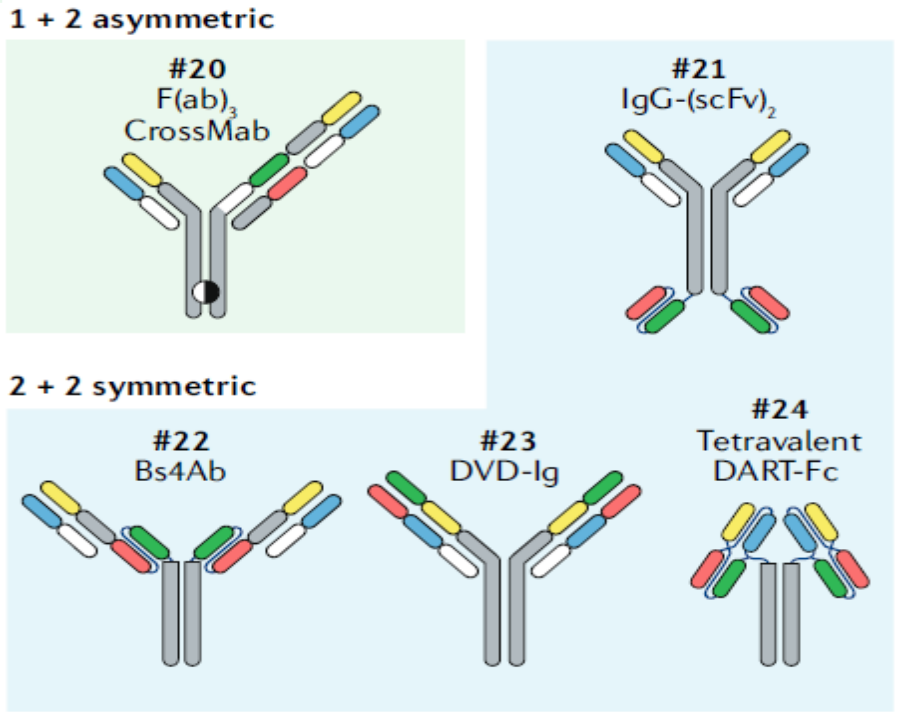
Considerations for bioassays and potency assays for novel ADC payloads

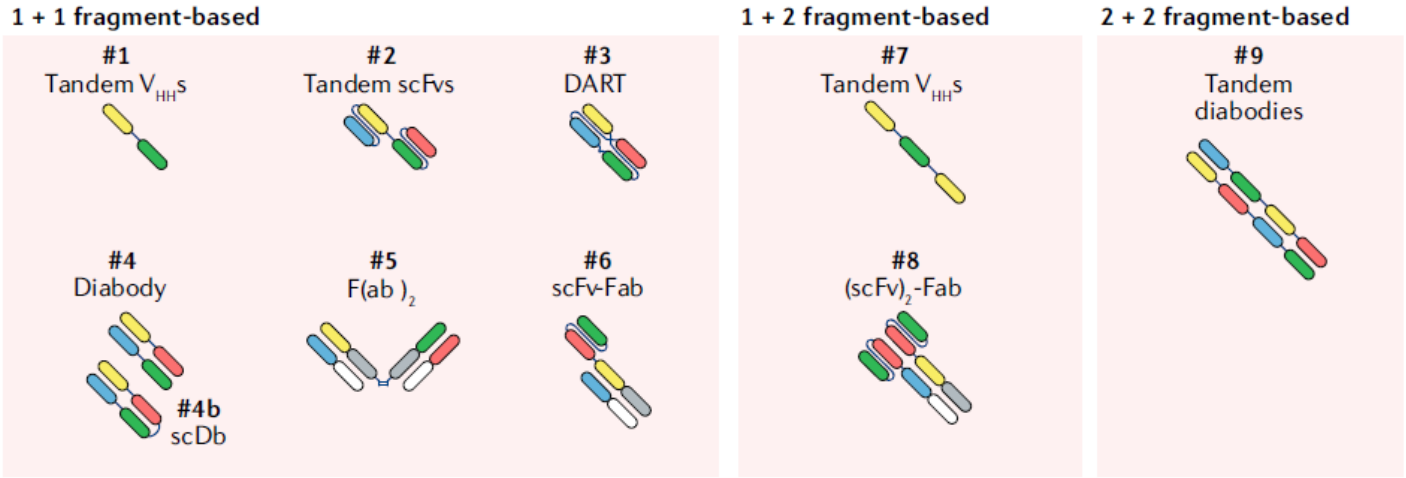
- TLR agonists
  - Activation of TLR agonist target effector cells
  - Release of cytokines by TLR agonist target cells
  - Antigen binding on target disease cells
  - mAb effector functions against target cells expressing antigen
- Oligonucleotides
  - Downregulation of target mRNA
  - Antigen binding on target cells
  - Characterization of mAb effector functions (lack of function)
- Antagonist
  - Assays appropriate for specific action of antagonist payload
  - Should be related to the intended action of the payload, such as cell death or inhibition of a specific pathway
  - Antigen binding on target cells

BsAb (multi-specifics) – many formats

- Ig Structure or appended Ig Structure
  - 1:1, 1:2 or 2:2 valency
  - Symmetric vs asymmetric
- Bispecific Fragments
  - With or without Fc
  - 1:1, 1:2 or 2:2 valency
  - Symmetric vs asymmetric
- Bispecific Fusion Proteins
- Bispecific Conjugates



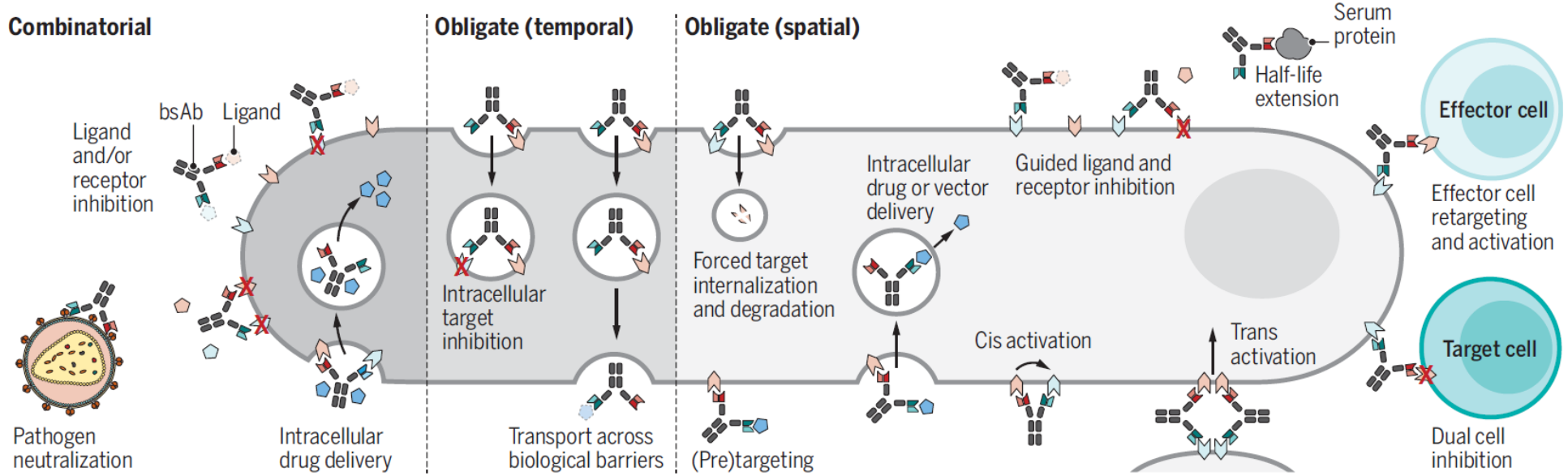




Labrijn AF et al., Nature Reviews Drug Discovery 18:585 2019

BsAb mechanisms of action

- Activating/inhibiting signal
  - Binding two antigens on a cell
  - Binding soluble ligands/pathogen molecules and preventing binding to receptor
- Recruiting effector cells
- Mimicking a co-factor
  - Emicizumab replaces Factor VIII
- Homing or shuttle
  - Delivery across the blood brain barrier



Brinkman U and Kontermann RE. Science. 372:916 2021

BsAb challenges for bioassays and potency assays

- Need to characterize the mechanism(s) of action as appropriate for the BsAb and indication
- May need multiple bioassays for characterization, need a justification for what should be used for release and stability assays
- Combinatorial MOA (each arm binding antigen independent of the other)
  - May not need an assay showing the BsAb can bind both antigens at the same time
- Obligate MOA (both arms need to bind ag)
  - May be sequential (shuttle construct) – may not need an assay showing can bind both ag at the same time, but need 2 assays showing binding to each target
  - May be a physical linkage – should have an assay showing both ag bound at the same time
- Fixed ratio of specificities – what is needed for 1+1, 2+ 2 or 1+ 2 designs?
- What downstream effects of binding need to be captured?
  - Is a potency assay needed for downstream effects?
- Is one assay sufficient?
- Do both antigens need to be bound by the same BsAb?

Bispecific Antibody Development Programs Guidance for Industry https://www.fda.gov/media/123313/download


It’s a multi-modal world

Bioassays for viral diseases: mAbs and hyperimmune globulins

mAb (treatment or prophylaxis)

- **Preclinical**
- Neutralization assay and/or effector function activity with live virus and/or pseudotyped virus/VLPs
- In vitro and in vivo
- Assess ability to neutralize different variants/strains/subtypes (SARS-CoV-2, rabies, HIV or influenza)

- **CMC**
- For potency assay, live virus or pseudotyped virus/VLP neutralization assays acceptable, based on BSL categorization of virus
- If mAb (or mAb cocktail) intended to neutralize different strains/subtypes (HIV/flu), justification for specific strains/subtypes used in potency assay
- Do not need to add new variants (SARS-CoV-2) to potency assay, provided mAb demonstrated to neutralize current circulating variants (live virus)
- May need assays to control effector function

Hyperimmune globulins (treatment or prophylaxis)

- **Preclinical**
- Neutralization assay with live virus or pseudovirus
- In vitro and in vivo
- Assess ability to neutralize range of variants (rabies) or new variants (SARS-CoV-2, influenza)

- **CMC**
- For potency assay, live virus or pseudovirus neutralization assays acceptable, based on BSL categorization of virus
  - Use of pseudovirus should be correlated with infectious virus assay
- Exception where neutralizing assay was challenging to validate (Hepatitis B)
- In some cases, ELISA for characterization of clinical lots (rare)
- Effector function usually for characterization, but not always
- Immune globulin potency testing requires neutralizing titers for measles and poliomyelitis Type 1, Type 2, or Type 3; new attenuated strain propose based on eradication of Types 1-3.
  - CBER standards are used for controls for these release tests when compared to reference material

See Guidances on Potency Assays for mAbs to treat COVID-19 and Developing mAbs for Rabies PEP

Bioassays for viral diseases: mAbs and vaccines

mAb (treatment or prophylaxis)

- **Preclinical**
- Neutralization assay and/or effector function activity with live virus and/or pseudotyped viral like particles
- In vitro and in vivo
- Assess ability to neutralize different isolates (rabies, HIV or flu) or new variants (SARS-CoV-2)

- **CMC**
- For potency assay, live virus or pseudotyped virus/VLP neutralization assays acceptable, based on BSL categorization of virus
- If mAb (or mAb cocktail) intended to neutralize different strains/subtypes (HIV/flu), justification for specific strains/subtypes used in potency assay
- Do not need to add new variants (SARS-CoV-2) to potency assay, provided mAb demonstrated to neutralize current circulating variants (live virus)
- May need assays to control effector function

Vaccines (prophylaxis)

- **Preclinical and clinical assess immunogenicity**
- Assess immunogenicity elicited by vaccine
  - B cell and/or T cell response
  - Qualified pre-clinical; fully validated post vaccination in clinical trials
- Assessment of functional immune response using in-vitro neutralization assay with live virus or pseudotype virus
- Assess ability to neutralize different isolates (rabies, HIV or flu) or new variants (SARS-CoV-2); update/revalidate

- **CMC**
- For potency assay, live virus or pseudotype virus neutralization assays acceptable, based on BSL categorization of virus
  - Validation prior to Phase 3 or testing Phase 3 samples
- Should be specific for virus, strain or variant against which immune response is measured
  - If vaccine modified for particular variant, assay should be updated
- Should have a link to immunogenicity or protection
- Release and stability assays should be strain specific

Even viral vectors for gene therapies have one potency assay similar to the other product types

mAb (treatment or prophylaxis)
Hyperimmune globulins (treatment or prophylaxis)
Vaccines (prophylaxis)

- Use **neutralization assays** to assess the ability of antibodies to neutralize **infectivity** of virus
- Product specific

Gene Therapies (treatment)

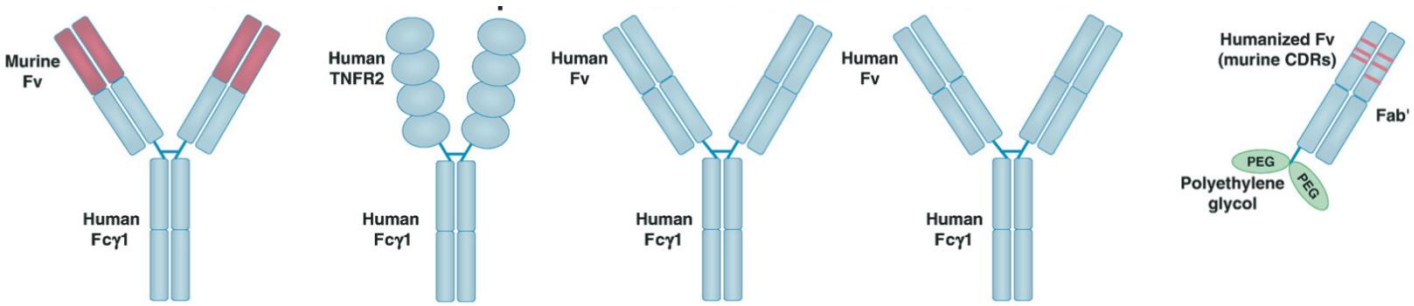
- **Infectivity assay** is used to determine virus titers
  - Frequently PCR based viral genomic titers are used to determine an infectious unit to genomic unit ratio
  - Infectivity assay variability an issue
- Similar approaches to select appropriate cell lines, establish growth conditions, and validate the assay.
- Product specific

- **Potency assays for anti-viral mAbs**
- Pretty good understanding of CQAs
  - Binding regions and PTMs
  - Glycans for effector function
  - Engineering to enhance or reduce effector function
  - Engineering for longer half-life
- Major MOAs should be understood – neutralization and/or effector functions
- May need additional assays to control effector function

- **Unique Challenges for Gene Therapy potency assays**
- CQAs not entirely understood
  - If Gene Therapy encodes a mAb, can use knowledge of mAb CQAs
- Complex MOAs and assays
  - MOA may not be fully understood
  - Needs to recognize target, sustain cell growth, intracellular signaling and kill target
- Limited time for testing if fresh cells used
- Need additional, appropriate assays, e.g., transgene expression, vector titer, assays for transfected cells (cytokine production, proliferation, lytic activity…)
- Potency assays may not correlate with in vivo efficacy due to cell expansion and persistence over time
  - Commonly accepted potency assay for CAR T cell products is a cytokine release assay (cytokine production upon stimulation by target)

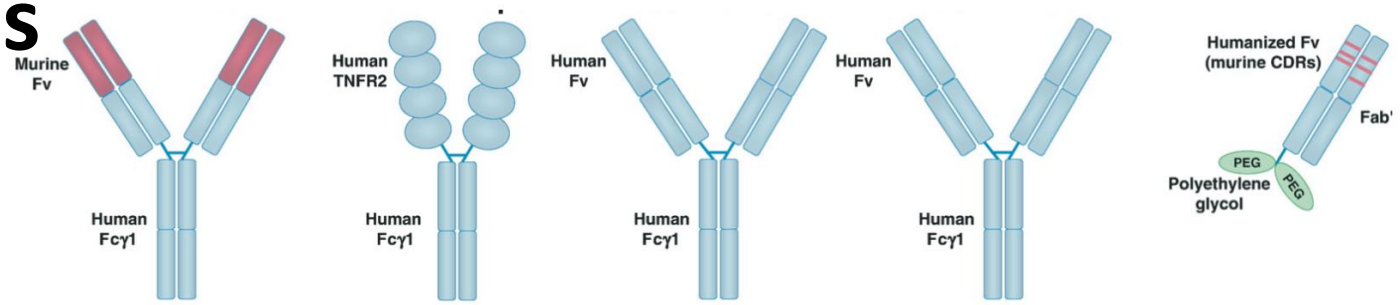

New Bioassays for Old Products

Approved TNF Antagonists



Clinical Indication	Infliximab	Etanercept	Adalimumab	Golimumab	Certolizumab
Class	IgG1	TNFR2	IgG1	IgG1	IgG1 / Fab
Origin	Chimeric mouse	Fc Fusion	Human Phage	Human	PEG
Molecular Weight	150	150	150	150	95
Specificity	TNF-α	TNF-α + LT-α LT-α2β1	TNF-α	TNF-α	TNF-α
	Trimer Monomer	Trimer	Trimer Monomer	Trimer Monomer	Trimer

Approved Indications



Clinical Indication	Infliximab	Etanercept	Adalimumab	Golimumab*	Certolizumab
Rheumatoid Arthritis	X	X	X	X	X
Juvenile Idiopathic Arthritis		X	X	X	
Ankylosing Spondylitis	X	X	X	X	X
Crohn's Disease	X		X		X
Pediatric Crohn's Disease	X		X		
Ulcerative Colitis	X		X	X	
Pediatric Ulcerative Colitis	X		X		
Plaque Psoriasis	X	X	X		
Pediatric Plaque Psoriasis		X			X
Psoriatic Arthritis	X	X	X	X	X
Pediatric psoriatic Arthritis				X	
Hidradenitis Suppurativa			X		
Uveitis			X		
Axial Spondyloarthritis					X

TNF Antagonist Potential MOAs

MOA	RA	AS	PsA	PsO	CD Pediatric CD	UC Pediatric UC
Blocking TNFR1 and TNFR2 activity via binding and neutralization of s/tmTNF						
	Yes	Yes	Yes	Yes	Likely	Likely
Reverse (outside-to-inside) signaling via tmTNF:						
Apoptosis of lamina propria activated T cells	-	-	-	-	Likely	Likely
Suppression of cytokine secretion	-	-	-	-	Likely	Likely
Mechanisms involving the Fc region of the antibody:						
Induction of CDC on tmTNF-expressing target cells (via C1q binding)	-	-	-	-	Plausible	Plausible
Induction of ADCC on tmTNF-expressing target cells (via FcγRIIIa binding expressed on effector cells)	-	-	-	-	Plausible	Plausible
Induction of regulatory MΦ in mucosal healing	-	-	-	-	Plausible	Plausible


Is a Bioassay always needed? Assessing potency for transition products, peptides and therapeutic proteins

Transition products

- Transition products approved as NDAs under section 505 of the FD&C Act were "deemed to be a BLA" as of March 23, 2020
- Similar products in clinical development, before or after March 23, 2020, are regulated as BLAs under the PHS act (351(a) or 351(k))
- NDA products assess "strength" rather than "potency"

Strength 314.3(b): the amount of DS contained in… a DP, which includes total quantity of DS in mass or units of activity… and concentration of the drug DS in mass or units of activity per unit volume or mass…

Potency 600.3(s): the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

Are there different requirements related to CMC that will apply to a biological product in a deemed 351(a) BLA?

- Yes, some differences, but expect many to be minimal due to similar types of considerations between product types
- May be required to report or provide different information than is required for biological products under the FD&C Act.

FDA may recommend changes to the control strategy throughout the product life cycle to modernize control strategies, to address product-specific issues, and to help ensure that biological products remain safe, pure, and potent

List of "Deemed BLAs" https://www.fda.gov/media/119229/download

Q&A Guidance https://www.fda.gov/media/135838/download

Do traditional BLA therapeutic proteins always use bioassays for release?

Enzymes regulated as BLAs, like those formerly regulated as NDAs, use enzymatic activity assays.

Enzyme replacement therapies include bioassays as part of characterization to demonstrate the enzyme can enter the cell

Thrombolytics use clot lysis assays

New peptides and proteins (formerly developed under Section 505) should include methods other than "assay" to assess potency.

Depending on the proposed mechanism, it could be a cell-based assay or cell-free assay

Even some mAbs may not need bioassays for release, for example:

- mAbs targeting bacterial toxins
- mAbs targeting endogenous extracellular targets that block an extracellular event
- Expectation that Fc effector functions be characterized (high, medium, low potential)
- In some cases where a bioassay would be appropriate and generally preferred, a cell-free assay may be acceptable if it is shown to be better or superior to the cell-based assay
  - The assay should reflect the proposed mechanism, such as inhibition of an activity
  - Consider using a cell-based assay as support for comparability

Regardless of product class, **<u>always</u>** discuss with the review team and provide data to support your position

Take Home Messages

- We’re never done learning about how our products work and the diseases they are used to treat
  - Accumulation of knowledge gained by industry and health authorities working together
- Continuous learning leads to new and improved bioassays, better products and hopefully better clinical outcomes
- Bioassays are important throughout a product lifecycle
- Close attention to developing bioassays during candidate selection and optimization lays the groundwork for bioassays used for pre-clinical assessments, CMC characterization, release and stability methods
- Potency assays and characterization methods should reflect the major MOAs and intentional design of the molecule
- Update methods for older products
- A bioassay for QC purposes may not be needed for some products
  - Where appropriate this may need a justification supported by data and discussion with the review team

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Stephan's Quintet as seen by Webb

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