Preclinical Drug Development of Biotechnology Derived Pharmaceuticals

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- President and founder of Expedient Solutions International, an international drug development, technology alliance and support company.
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- Co-founder of Bridge Laboratories (now Pharmaron in Beijing, China) and built the first Western standard GLP and AAALAC accredited preclinical lab in China
- Translational Medicine: Product development and project management expertise to bring drug candidates (biotech protein therapeutics, peptides and vaccines, small molecules and botanical products) from discovery research to preclinical and clinical.
- Experience in numerous therapeutic areas: anti-cancer, anti-infective/antimicrobial, hematological disorder, neurologic disorder, HIV, autoimmunity/immune related disorders, anti-inflammatory, organ transplantation, stem cell and allergy
- Instrumental in the completion of submission of IND's with anticancer small molecule drugs, cytokine and botanical products. Submitted the first two IND's with botanical products for China-based company. Support for post-IND technical and regulatory up to NDA and BLA submissions
- Experience in the EC CTX and CPMP regulatory document preparations. Represented companies to present before FDA and various regulatory authorities.

Part I: Principles (including ICH Guidelines) and Practices

ICH Guidelines (S6): Preclinical Evaluation of Biotechnology-Derived Pharmaceuticals (1997)

- Case-by-case, Science-based approach
- Product used in Pharm/Tox studies comparable to product for initial clinical studies: Formulated product vs API
- Biological Activity, Receptor binding, Cross- reactivity in man & a range of animal species
- Animal Species: Pharmacologically active *in vitro/in vivo*: epitope or receptor expression; transgenic animals expressing human receptors or homologous proteins
- Number of animals (increases in frequency & monitoring duration)/Gender
- Administration/dose selection: Route/frequency approximates clinical use, Doses of multiples of human dose, PK, NOAEL, MTD/MFD
- Immunogenicity: Antibody titers, Antibody influence on PK/PD, complement activities and pathology

ICH Guidelines (S6): Addendum to Preclinical Evaluation of Biotechnology-Derived Pharmaceuticals (2012)

- One or two species for longer term toxicity studies. One species is sufficient provided the toxicological findings from these studies are similar in different species
- Dose selection: High dose selection should be the higher of two doses when a dose provides the Maximum intended pharmacological effect and a dose provides an approximately 10-fold exposure multiple over the max exposure to be achieved in the clinic.
- Dose selection: large relative difference in binding affinity or in vitro potency.
- Duration: Chronic use products: Repeat dose toxicity studies of 6 months in both rodent and non-rodent should be sufficient
- Recovery: reversibility of toxicity Not the delayed toxicity. Complete recovery not needed. Recovery to assess the immunogenicity not required.

ICH Guidelines (S6): Addendum to Preclinical Evaluation of Biotechnology-Derived Pharmaceuticals (2012) (cont.)

- Measurement of anti-drug (ADA) should be evaluated if there is (1) altered PD; (2). Unexpected changes in exposure in the absence of PD marker and (3) evidence of immune-mediated reactions (immune complex disease, vasculitis, anaphylaxis….etc)
- Reproductive and Developmental toxicity: S5(R2) detection of toxicity to reproduction for medicinal products and toxicity to male fertility (ICH S5):follow the same principles for species, dose level selection…etc
- Fertility: ICH S5. If NHP is the only relevant species: evaluation of reproductive organs in repeated dose tox study with 3-month duration. It is not recommended to produce a homologous product or transgenic model solely to conduct mating studies in rodents.
- Timing of the repro studies: prior to phase III
- May not need carcinogenicity except for Immunosuppressives and growth factors, weight of evidence

Animal Species

- MAB: Cross-reactivities with the target cell and adverse effect as a result of undesirable binding to tissues – immune complex formation (Pathology)

  in vitro cell-based assay and in vivo efficacy, Immunohistochemistry
- Cytokine: Exhibit comparable pharmacologic effect as in man; similar protein sequence

Parameters to be Evaluated

- Immunogenicity and anti-drug antibody titers
- Immunotoxicity:
  - -Inflammatory reactions at the injection site
  - -Distribution of surface antigen on target cells, e.g. CD4/CD8 ratios on T cells and CD20 for B cells
- Immune Function Assays: Natural killer cell activity, Host resistance, Macrophage/Neutrophil function
- If targeting CD3 on T cell: Cytokine storm

Parameters Potentially Affecting the Immunogenicity

- May lose efficacy
- Presence of protein aggregate potentially increases immunogenicity
- Subcutaneous (vs iv) injection more likely induce immunogenicity
- The presence of foreign protein (non-self)
- MAB targeted at cell bound epitope ~Immune stimulating protein
- Pegalation decreases immunogencity
- Impaired immune system in cancer patients
- Modified Fc may be less immunogenic

Additional Considerations for Study Design for Proteins

- Recovery group:
  - -Evaluate reversibility (or the trend of reversibility) of toxicities
  - -Allow more time for the antibody titer generation
- Metabolism and gene tox may not be needed
- Local Tolerance (can be formulation)
- Case-by-case

  Reproductive: Clinical indication & intended patient population: Tysabri for MS

  Chronic and carcinogenicity: Generally inappropriate (growth factors)
- PK/TK
- Safety Pharm in one species
- Immune complex formation

Other Issues with MAB & Proteins Preclinical Development

- Dose response may be threshold related and not dose dependent
- Toxicity of excipient components. GRAS list.
- Protein aggregates and degradation products
- Bioassay and cell-based assay for drug release, tertiary structure, potency
- Exaggerated pharmacology vs frank toxicity
- Formulation may affect immunogenicity and bioavailability and the selection of route of administration (SC vs IV)

Immunopathology: Cross-Reactivity with Normal Human Tissues

- FDA Points to consider in the manufacture and testing of monoclonal antibody products for human use, Feb 28, 1997.
- Immunohistochemistry in quick-frozen adult human tissues (tissue cross-reactivity with human tissues)
- At least 3 unrelated human donors
- Several concentrations of product to be used: Cmax
- Positive (anti-transferrin receptor Mab) and negative controls
- Same as TCR tissue cross-reactivity

Estimation of Clinical Start Dose-Protein Drugs

- No fixed algorithm, case-by-case, and risk-benefit conditions
- MABEL: Minimum Anticipated Biological Effect Level
- PAD: Pharmacologically Active Dose
- MRSD: Maximum Recommended Starting Dose
- NOAEL, NOEL
- HED: Human equivalent dose from NOAEL, NOEL

  MABEL~/= PAD ~= NOEL, or MRSD
- HNSTD: Anti-cancer drugs

Estimation of Clinical Start Dose (Cont.)

- In vitro receptor affinity and density data
- AUC vs mg/kg
- Application of a safety factor:
  - PK-based interspecies scaling
  - Weight or surface area dose normalization
  - Known safety of other proteins with similar MOA
  - Can be 10X to 50X

cGMP for Phase 1 Investigational Drugs

- Guidance for Industry: cGMP for Phase 1 Investigational Drugs, July, 2008.
- The GMP requirements continually increase as the drug development moving from preclinical to phase 1, 2, 3.
- Intend to streamline and promote the drug development process while ensuring the safety and quality of the earliest stage investigational drug products-Phase 1.

  --Use disposable equipment and process aids to reduce bioburden

  --Prepackaged WFI & sterilied containers to eliminate qualifying existing equipment

cGMP for Phase 1 Investigational Drugs (cont.)

- Use of closed process equipment to eliminate stricter room classification for air quality: under laminar flow hood vs class 100 room.
- One product manufactured in an area/room at a time
- Documentation is streamlined –Batch records not necessary

Toxicology Studies Post IND Submission

- Species and Route: same as pre-IND studies: small molecules: rat/dog; protein and vaccine: rat/NHP
- Dosing Regimen and Duration: match the clinical
- Reproduction and Teratology: in 2 species toward mid and late Phase II clinical
- Start planning and initiating tox studies to support Phase III
  - Chronic: 6 month in rodent or non-rodent
  - Carcinogenicity: Rat or transgenic mouse model

Part II: Special Products: Cytokines, Monoclonal antibodies, Bi-specific antibodies, ADC, Oligonucleotides as well as Biosimilar products

Cytokine

Neulasta (Peg-GCSF (2002)

- Pharmacology: in vitro and in vivo
- PK/PD: single dose in rat and NHP
  - PK: Clearance of Neulasta
  - PD: Effect on ANC (Absolute Neutrophil Count)

Neulasta (Peg-GCSF): Toxicology

- Single dose IV for 2 weeks toxicity in rats
- Two-week SC toxicity study in rats: 0, 50, 100, 500, 1000 ug/kg: q2d
  - All dose groups: ↑neutrophil, WBC,
  - 500 ug/kg and higher: lymphocyte
  - ↓red cell parameters
  - dose-related ↑ AlkP, AST Ca ↓ K
  - Saturated TK
  - ↑spleen (≥ 100 ug/kg), liver (1000 ug/kg) and prostate (≥100 ug/kg) wt
  - Histopath: BM, Spleen, liver, bone, lung, LN
  - ADA in all three dose levels
  - NOEL: 100 ug/kg

Neulasta (Peg-GCSF): Toxicology

- One-month repeat dose SC toxicity with one-month recovery in Cynomolgus monkeys: 0, 75, 250, 750 ug/kg, qiw x 5
  - All findings similar to the 2-week study
  - NOAEL: 750 ug/kg
- 3/6 month SC/IV toxicity study in rats with recovery: NOAEL: 1,000 ug/kg (SC) & 300 ug/kg (IV), qiw x 3/6 months

Neulasta (Peg-GCSF): Toxicology

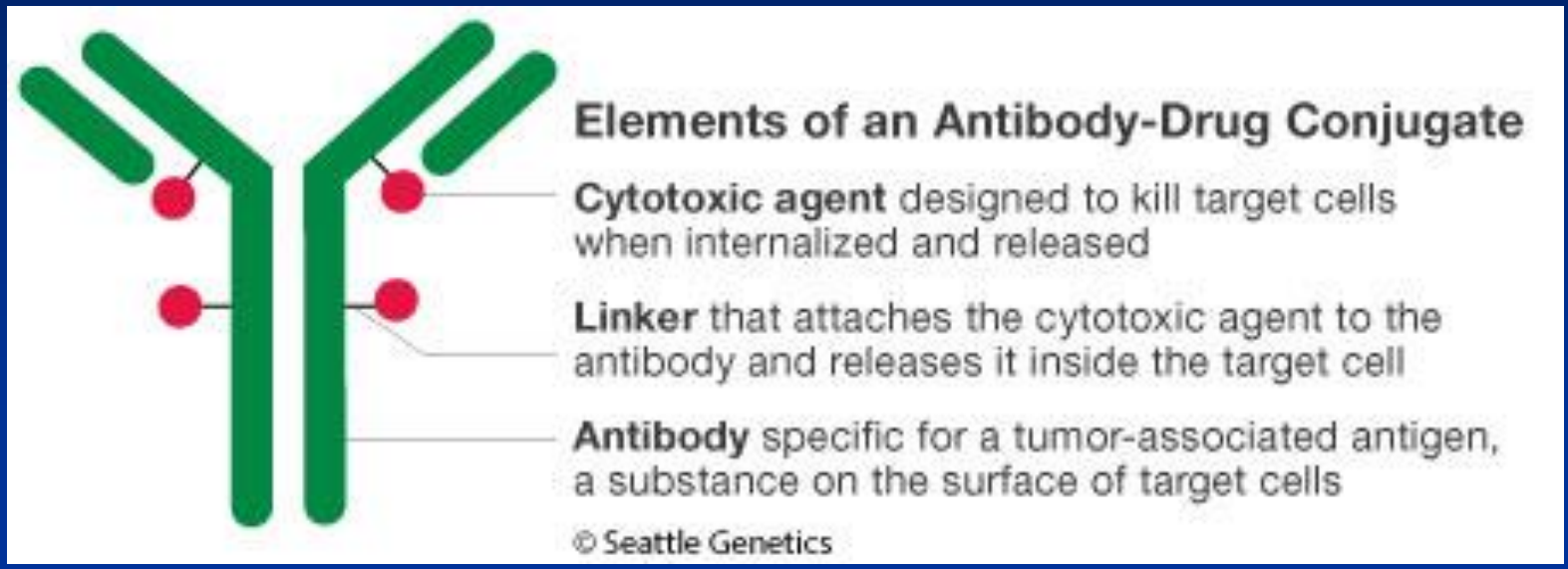
- Reproduction and Teratology:
  - Embryo/fetal: rabbit and rat
  - Fertility and early embryonic
  - Pre- & Postnatal development, including maternal function in rats

- Safety pharmacology studies: Part of Repeat dose tox studies
- No mutagenicity
- Carcinogenicity: No evidence that G-CSF administration results in tumor formation.

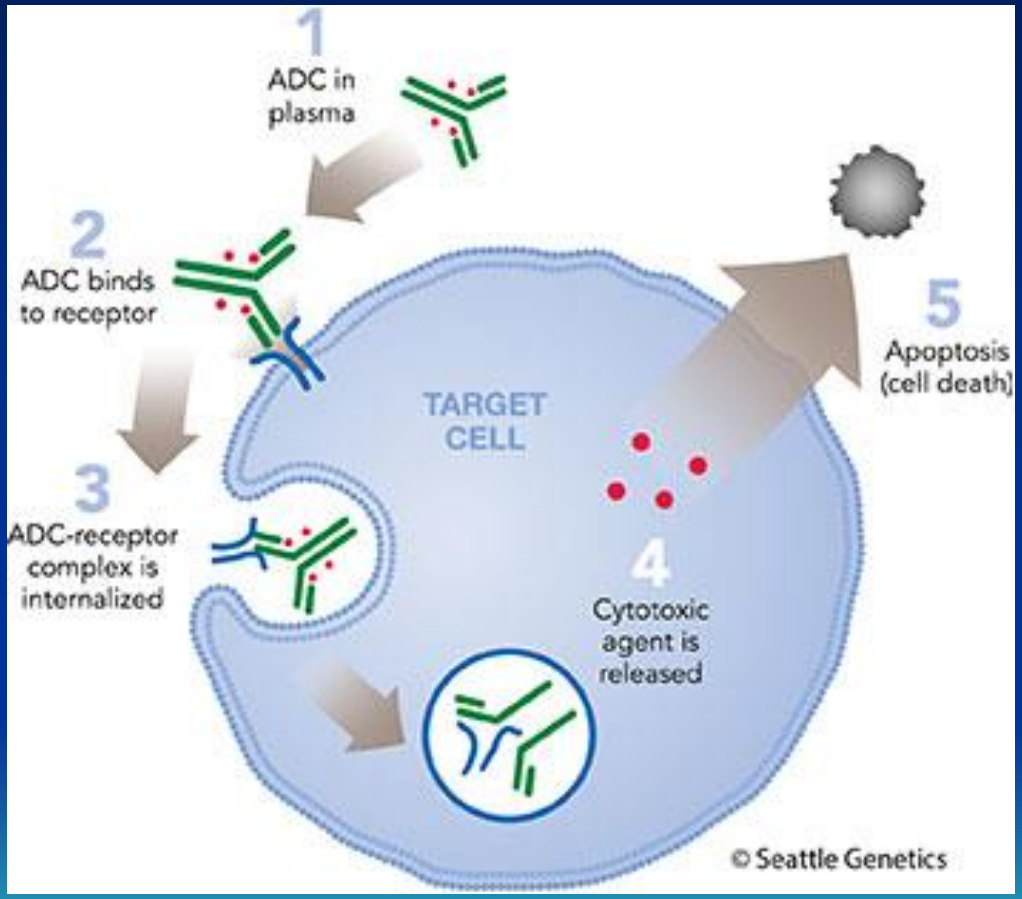
Antibody-Drug Conjugate (ADC)

Structure of ADC

- Antibody:Human IgG isotypes.
  - Antibody dependent cellular cytotoxicity (ADCC)
  - Complement dependent cytotoxicity
- Linker and conjugation:
  - Linker should be stable; not to release the cytotoxic drug before reaching its target and cause off-target toxicity
  - Linker should be able to release the drug efficiently once internalized
  - DAR: Drug-antibody ratio
- Cytotoxic payload:
  - Traditional chemotherapy drugs: doxorubicin, methotrexate


Elements of an Antibody-Drug Conjugate
Cytotoxic agent designed to kill target cells when internalized and released
Linker that attaches the cytotoxic agent to the antibody and releases it inside the target cell
Antibody specific for a tumor-associated antigen, a substance on the surface of target cells
© Seattle Genetics

Primary Mechanism of Action of ADCs: Targeted Delivery of a Potent Cytotoxic Agent



Mechanism of ADC Toxicity

	Mechanism(s)	Safety assessment
On-target	Binding/internalization of ADC in target-expressing normal cells	*In vivo* toxicity must be evaluated in crossreactive species; requires understanding of normal tissue target expression
Off-target	Instability of conjugate; nonspecific uptake into normal cells (*i.e.*, Fcγ receptors, FcRn binding, pinocytosis, *etc.*); nonspecific binding of antibody to normal cells	Off-target toxicity can be evaluated in non cross-reactive species

Minimal Requirements for IND/BLA

Study	Molecule	Study duration	IND	BLA
GLP toxicology in relevant species (incl. safety pharmacology endpoints)	ADC	Single or repeat dose[a]	√	
		3 months		√
GLP toxicology study in rat[b]	Warhead	Single dose	√	
In vitro plasma stability	ADC	N/A	√	
Tissue cross-reactivity	ADC	N/A	√	
Genotoxicity battery[b]	Warhead			√
Embryofetal development	ADC			√[c]

Minimal Requirements for IND/BLA (cont.)

- [a]Refer to ICHS9 for additional details on study duration required to support IND applications
- [b]If warhead has been previously tested and sufficient body of scientific information is available, a separate evaluation is not warranted
- [c]If warhead is genotoxic and targets rapidly dividing cells, an embryofetal development toxicology study is not warranted (refer to ICHS9)

- *From "Antibody Drug Conjugate: Nonclinical Safety Consideration"; AAPS Journal 2015 Sep 17(5): 1055-1064*

FDA Approved ADC
(SBA: summary basis of approval from FOI)

- T-DM1, Kadcyla: Anti-HER-2 AB linked to DM1. For HER2-positive metastatic breast cancer. (2013)
- Brentuximab vedotin Adcetris: Anti-CD30 mAB connected with a cleavable peptide to the highly potent tubulin inhibitor MMAE. CD30 is a member of TNF family. For Hodgkin lymphoma. (2011)
- Gemtuzumab ozogamicin, Mylotarg: Humanized IgG4 mAB directed against CD33, a surface antigen present in 90% of AML (Acute myelogenous leukemia) linked to calicheamicin cytotoxin. (2017)
- Inotuzumab ozogamicin: Humanized mAB against CD22 linked to a cytotoxic agent from the class of calicheamicins called ozogamicin. For relapsed or refractory B-cell precursor acute lymphoblastic leukemia. (2017)

Besponsa, CMC-544 (2017)

- ADC consisting of a humanized CD22-directed IgG4 monoclonal antibody (inotuzumab, G544) and a semi-synthetic derivative of the cytotoxic natural product
- CD22-directed cytotoxicity against cancer cell line of B cell origin while sparing the CD22-negative AML.
- In vitro and in vivo efficacy (xenograft ALL mouse model)
- Safety Pharmacology: CV: small and transient effect on arterial blood pressures in monkey. CNS and respiratory: no toxicity in male rats
- Toxicology: IV, 1X/wk for 4 wks in rats at 5, 15, 50 ug/kg and monkeys at 2.5, 8, and 25 ug/kg.
  - -Similar toxicity in rats and monkeys
  - -↓ body weight/food consumption, ↓ RBC,HGB, HCT, Lym
  - -↑ ALT, AST, AP:hepatotoxicity, toxicity at male reproductive organs

Besponsa, (CMC-544) (Cont.)

- Toxicology (1). IV, 1X/wk for 26 wks at 1, 3, 10 ug/kg:
  - 10 ug/kg: ↓ body weight and food consumption
  - Adverse effect at liver and testes at ≥ 1 ug/kg including hepatocellular adenomas at 10 ug/kg. (2). IV, 1X/wk for 26 wks at 0.5, 1.5, 5.0 ug/kg:
  - 5 ug/kg: three monkeys were prematurely sacrificed at moribund
  - Target organs were liver, ovary, hematolymphopoietic tissues: spleen, mesenteric lymph node, thymus.
- Genetic toxicology:

  AMES, in vitro and in vivo micronucleus: all positive
- Carcinogenicity: Not done (ICH S6, S1 and S9)
- Reproductive and Development Toxicology

  -Fertility and early embryonic in female rats: 0.15, 0.5, 1.5 ug/kg, IV, once daily: Maternal and embryonic toxicity at 1.5 ug/kg, NOAEL:0.5 ug/kg

  -Embryo-fetal in rats: Maternal and fetal toxicity:1.5 ug/kg,NOAEL:0.5

  -Embryo-fetal in rabbits: 0.1,0.3, 1.0 ug/kg, IV daily X14 days: slight maternal toxicity at 1 ug/kg; NOAEL for developmental tox: 1 ug/kg

Besponsa, (CMC-544) (Cont.)

- Other studies:
  - Cross-reactivity with normal human tissues:
    - G-544 stained the membrane and cytoplasm of lymphocytes consistent with know pattern CD22 expression
    - Unexpected G544 stained the stromal fibers in the dermis of the skin, mammary gland and uterus cervix

Immunotherapy: Bi-Specific Monoclonal Antibody

Cancer Immunotherapy

The use of immune system to treat cancer

- Antibody:

  Bind to cancer antigen; CD20, CD274, CD279

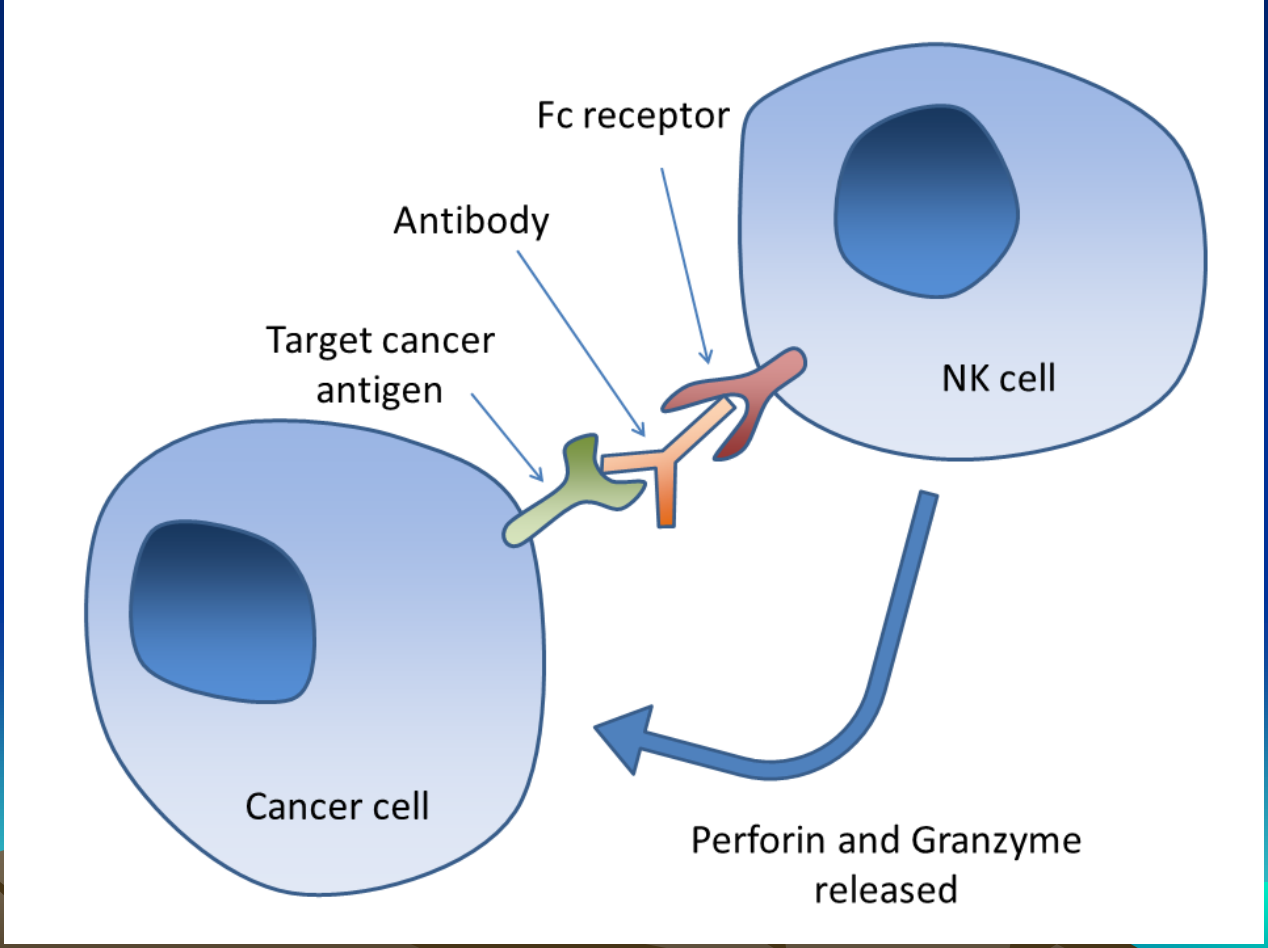
  ADCC (Antibody-dependent cell-mediated cytotoxicity or activate complement system or prevent a receptor from interaction with its ligand→Death.

- Cellular therapy:

  Cancer vaccine: removal of immune cells from blood or from tumor activated, cultured return to patient. Immune cells attacks the cancer: NK cells, lymphokine-activated killer cells, cytotoxic T cells and dendritic cells: Provenge from Dendreon for prostate cancer.

- Cytokine: IL-2 & interferon-α

ADCC: When the Fc receptors on NK interact with Fc regions of antibodies bound to cancer cells, the NK releases perforin and granzyme, leading to apoptosis



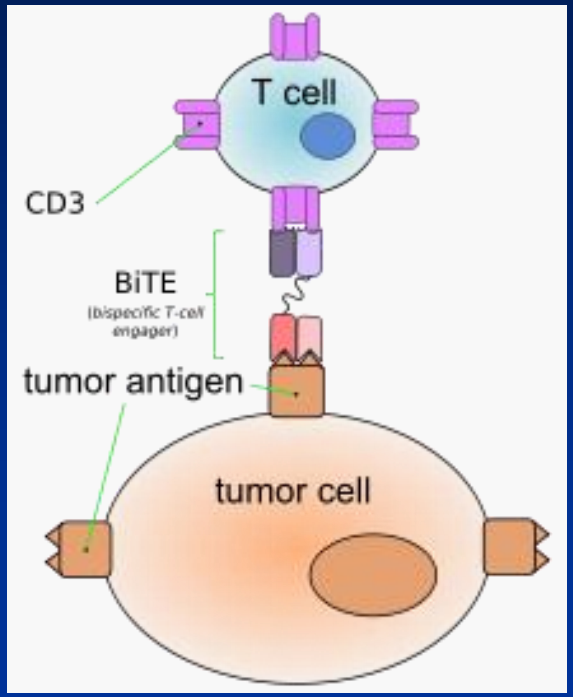
Perforin and Granzyme

- Perforin:
  - It is a protein
  - Creates transmembrane tubules and is capable of lysing non-specifically a variety of target cells
  - One of the main cytolytic proteins of cytolytic granules
  - Key effector molecule for T-cell and natural killer-cell-mediated cytolysis

Perforin and Granzyme (cont.)

- Granzyme
  - Serine protease
  - Released by cytoplasmic granules within cytotoxic T cells and natural killer (NK) cells
  - Induce programmed cell death in the target cell
  - Eliminate cells that have become cancerous
  - Packaged in cytotoxic granules with perforin
  - Mediates the delivery of granzymes into endosomes in the target cells

Blinatumomab: Against CD19 on B cells and CD3



Blinatumomab linking a T cell (CD3) to a malignant B cell (CD 19)

BiTE:Bispecific T-cell engager

First bispecific antibody for ALL (Acute Lymphoblastic Leukemia)

History

- July 2014: Granted breakthrough therapy
- October 2014: BLA was granted priority review
- Dec 3 2014: Approved by FDA

FDA Guidance for Industry Expedited Programs for Serious Conditions - Drugs and Biologics (May 2014)

- To facilitate and expedite development and review of new drugs to address unmet medical need in the treatment of a serious or life- threatening condition.

- Four expedited programs:
  1. Fast track designation
  2. Breakthrough therapy designation
  3. Accelerated approval
  4. Priority review designation

Pharmacology of Blinatumomab (BiTE)

- Primary Pharmacology
  - Binds to B (CD20) and T (CD20) and T (CD3) lymphocyte in PBMC of Chimpanzee but not to mouse, dog, Cyno monkey, rhesus monkey and baboon via FACS
  - Binding of BiTE by T lymphocytes from Chimp resulted in redirected lysis of B lymphocytes by T cells in PBMC by FACS

Pharmacology of Blinatumomab (BiTE) (cont.)

Mechanism of action:

- BiTE-mediated cytotoxicity mediated by perforin
- BiTE treated PBMC: No T cell proliferation noted in PBMC depleted of B cell
- BiTE-mediated redirected lysis was directly correlated with CD19 expression on target cells, resulting in increased activation markers CD25 and CD69 and cytokine release.
- Pre-treatment of PBMC with dexamethasone resulted in inhibition of cytokine release

Pharmacology (cont.)

- Selection of relevant animal species:
  - Cross-react with CD20 (19) Lymphocytes from Chimp, not from mouse, dog, rat, monkey, and baboon (in PBMC)
  - Murine surrogate (or homologous), muS103new for in vitro bioactivity
  - All toxicology studies were conducted using muS103new in rodents.

Toxicology of muS103new
(murine surrogate bi-specific antibody)

- Repeat-Dose Toxicity:
  1. Once daily IV for 4 weeks + 4 weeks recovery: 0.2, 1, or 5 mg/kg/day in BALB/c mice
  2. Twice daily SC for 13 weeks + 4 weeks recovery:0, 2, or 10 mg/kg/dose in BABL/c mice

Toxicology of muS103new
(murine surrogate bi-specific antibody) (cont.)

- ↓WBC, Lymphocyte: depleted B and T cells, NK cells
- ↓ Spleen weight
- ↓ Cellularities of lymph nodes

Toxicology of muS103new
(murine surrogate bi-specific antibody) (cont.)

- No genetic toxicology studies
- No Carcinogenicity
- Reproductive and Developmental Toxicology
  - No fertility and early embryonic development studies were submitted
  - Only embryonic fetal development in mice via IV: 0 and 5 mg/kg

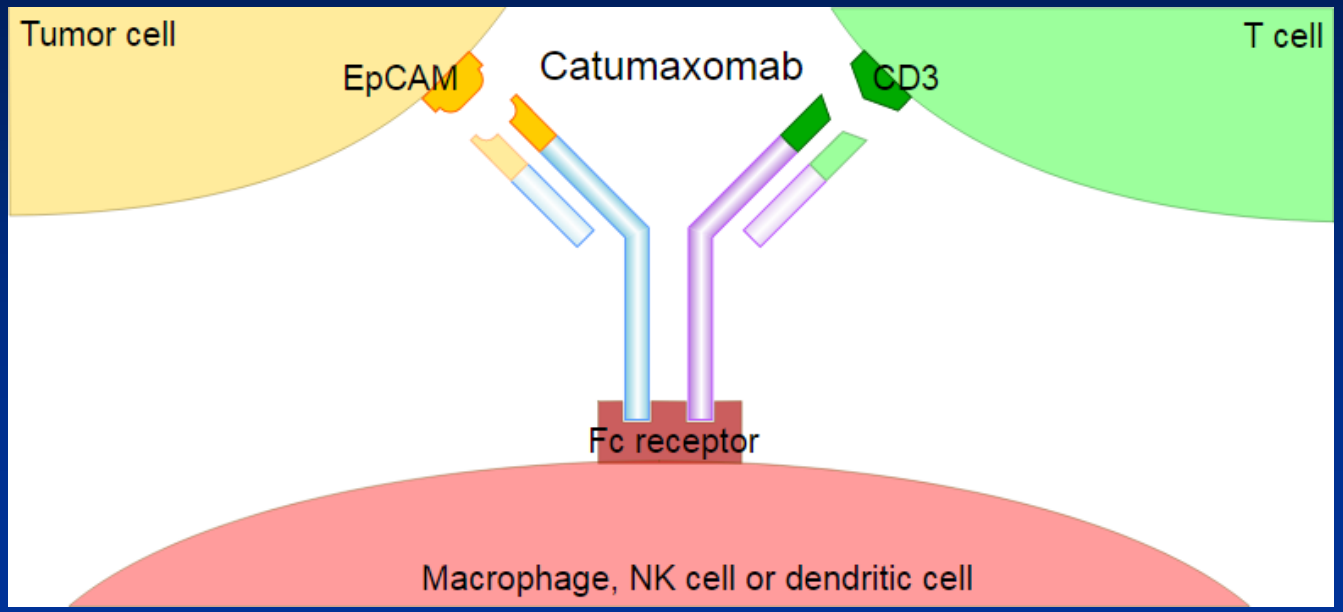
Toxicology of muS103new
(murine surrogate bi-specific antibody) (cont.)

- Parameters evaluated in addition to general evaluation in the reproductive tox study:
  - CD4/CD8
  - Activated CD4/CD8
  - Total T cells, B cells, NK cells, activated T cells

Safety Pharmacology

- MuS103new : Murine surrogate behaves the same as BiTE
- Irwin Test : Behavior Test (FOB)
- Dose Levels: 0, 0.2, 1 or 5 mg/kg/day x 5 days, IV bolus: slight CNS effects at 1 mg/kg

Catumaxomab: Against CD3 and EpCAM (2009 by EMA)



- Catumaxomab linking a T cell (CD3) to a tumor cell (EpCAM)
- A rat-mouse hybrid monoclonal antibody
- Treat malignant ascites

Pharmacology of Catumaxomab

- *In vitro* effects of catumaxomab were assessed in models using human cells where catumaxomab is able to exert its full pharmacological activity.
- A surrogate antibody (BiLu), which is of equivalent structure to catumaxomab and has the same principal target specificity but binds to mouse CD3 instead of human CD3, was used for pharmacology, pharmacokinetic and toxicology studies in mouse models.

Pharmacology of Catumaxomab (cont.)

- *In vitro*
  - Specificity of binding of catumaxomab to CD3 and EpCAM
  - Determination of the antibody binding site on EpCAM
  - Binding properties of catumaxomab to various human tumor cell lines. Anti-tumor activity against human tumor cells from different tissue
  - Binding of catumaxomab to human Fcγ receptors of monocytes, NK cells and granulocytes
  - Activation of human T-cells by catumaxomab
  - Antibody-dependent cellular phagocytosis
  - Complement-dependent cytotoxicity

Pharmacology of Catumaxomab (cont.)

- *In vivo*
  - a syngeneic mouse tumor model expressing human EpCAM
  - a human ovarian carcinoma xenograft study

Pharmacology of Catumaxomab (cont.)

- Mechanism of action
  - The Fc-region of catumaxomab enables interaction with accessory immune cells via Fcγ receptors.
  - Thereby, a concerted immunoreaction against tumour cells is induced which includes different mechanisms of action such as T-cell activation, antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and phagocytosis. This results in destruction of tumor cells.

Toxicology of Catumaxomab

- Tissue cross reactivity: mouse, monkey, rabbit and dog
- Single dose toxicity: 3 monkeys marmoset, cynomolgus and rhesus, **no acute toxicity was seen even at very high doses**
- Antigenicity: Single dose toxicity study in the cynomolgus monkey. The development of anti-mouse and anti-rat antibodies was detected in serum samples after the last dose

Toxicology of Catumaxomab (cont.)

- Local tolerance: reversible erythema formation in rabbit i.a. and s.c.; no erythema or edema formation via i.m.
- Immunotoxicity: Single dose toxicity study in the cynomolgus monkey. No changes were seen in the serum cytokine levels following i.v. infusions of catumaxomab at escalating dose levels. In addition, no influence on the plasma levels of complement was observed.
- No Repeat dose toxicity, genotoxicity, carcinogenicity, reproduction toxicity

- **Biosimilar**

Guidance for Industry: Quality Considerations in Demonstrating Biosimilarity of a Therapeutic Protein Product to a Reference Product (2015)

- Expression System
- Manufacturing Process
- Assessment of Physicochemical Properties
- Functional Activities
- Receptor Binding and Immunochemical Properties
- Impurities
- Reference Product and Reference Standards
- Finished Drug Product
- Stability

Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (2015)

- Complexities of protein products
  - Nature of protein products and related scientific considerations
  - Manufacturing process considerations
- U.S.-Licensed reference product and other comparators
- Approaches to developing and assessing evidence to demonstrate biosimilarity
  - Using A stepwise approach to demonstrate biosimilarity
  - Using A totality-of-the-evidence approach to assess A demonstration of biosimilarity

Guidance for Industry: Scientific Considerations in Demonstrating Biosimilarity to a Reference Product (cont.)

- Demonstrating biosimilarity
  - Structural analyses
  - Functional assays
  - Animal data
    - Animal toxicity studies
    - Inclusion of animal PK and PD measures
    - Interpreting animal immunogenicity results

FDA Approved Biosimilars

Drug Name		Approval Date
Zarxio	(Filgrastim-sndz)	March 2015 (FDA first biosimilar)
Inflectra	(Infliximab-dyyb)	April 2016
Erelzi	(Etanercept-szzs)	August 2016
Amjevita	(Adalimumab-atto)	September 2016
Renflexis	(Infliximab-abda)	May 2017
Cyltezo	(Adalimumab-adbm)	August 2017
Mvasi	(Bevacizumab-awwb)	September 2017 (FDA first biosimilar for the treatment of cancer)
Ogivri	(trastuzumab-dkst)	December 2017 (FDA first biosimilar for the treatment of certain breast and stomach cancers)
Ixifi	(infliximab-qbtx)	December 2017

FDA → Drug → Biosimilar Product Informati

European Medicines Agency

- Bioidentity Tests
  - In vivo Bioidentity
  - In vitro Cell-based Bioidentity
- Toxicological Studies
- Clinical Studies
- Pharmacovigilance Plan
- Analytical Procedures

Oligonucleotides

Oligonucleotides

- No FDA published Guidance.
- DNA antisense, RNA…etc.
- FDA approved products:

CMC and Preclinical

- Production Methods:
  - Synthetic oligonucleotides: similar to small molecules
  - Fermentation: similar to protein products
- Preclinical:
  - FDA Guidance: M3 (R2)
  - Biodistribution: PCR
- Analytical/Bioanalytical:
  - Hybridization (probe)
  - LC-MS

FDA approved oligonucleotide drugs

Drug	Nusinersen	Fomivirsen Sodium	Mipomersen sodium Injection	Pegaptanib sodium injection	Exondys 51	Defibrotide
Trade name	Spinraza	Vitravene	Kynamro	Macugen	Eteplirsen	Defitelio
Code Name	ISIS 396443	ISIS 2922	ISIS 301012			
Approval	2016	1998 (US), 1999 (EU)	2013	2004	2015	2013 (EU), 2014 (US)
Company	Biogen	ISIS (ONIS) Pharmaceuticals, Inc.	Genzyme	OSI Pharmaceuticals	Sarepta Therapeutics Inc.	Jazz Pharmaceuticals
Indication	Spinal muscular atrophy	Cytomegalovirus Retinitis (CMVR) in AIDS patients	Homozygous Familial Hypercholesterolemia	Neovascular (wet) age-related macular degeneration	Duchenne Muscular Dystrophy	Veno-occlusive disease in the liver of bone marrow transplant

Nusinersen (ISIS 396443)

- RNA: antisense oligonucleotide: fully 18-base 2'-MOE phosphorothioate oligonucleotide that was designed to be complementary to and hybridize with pre-RNA for human SMN2 to increase exon 7 inclusion and full length SMN protein expression
- Indication: SMA: Spinal muscular atrophy
- Pharmacology:
  - In vitro: splicing assay, reporter gene assays and SMA patients
  - In vivo: Humans are the only species known to have the SMN2 gene, therefore using transgenic mouse model. Single ICV (intro cerebral ventricular) injection → ↑ SMN genes.

Nusinersen (ISIS 396443)

- Safety Pharmacology: continuous intrathecal infusion (minipump) in rats: 0.02, 0.06, 0.2 mg/kg for 28 days.
  - No effects in respiratory, cardiovascular
- Pharmacokinetics:
  - Single bolus in adult monkeys
  - 14-week IT in juvenile monkeys
  - 53-week IT in juvenile monkeys
  - Absorption, Distribution and Metabolism and Distribution
- Toxicology:
  - Single dose IT bolus in monkeys: 0, 1, 3, 7 mg to adult monkeys
    - Clinical observations, body weight, physical and neurological exam, Clinical pathology, histopathology
  - Apparent acute neurological impairment after dosing in a single male

Nusinersen (ISIS 396443)

- 14-week multiple dose, IT bolus in juvenile monkeys + 4 week interim sac + 12 weeks recovery: 0, 0.3, 1, 3 mg/dose once weekly for 15 doses (day 99).
  - No mortality, no effects on body weight, clinical pathology, ophthalmoscopy, cardiovascular functions except for neurological changes at HD.
  - Acute neurological deficits at HD and brain histopathology at MD1
  - TK: blood and CSF, tissue concentration, hippocampus concentration
- 13-week subcutaneous injection toxicity study in juvenile CD-1 mice: once weekly from PND 4 to 25 and once every other week through PND 95 at 0, 1, 10 or 50 mg/kg.
  - Renal, hepatic, spleen, bone marrow and injection site findings and MD and HD
  - TK

Nusinersen (ISIS 396443)

- One year repeat dose toxicity study in juvenile monkeys with a 26-week recovery phase at 0.3, 1. 4 mg/dose IT administration in juvenile monkeys
  - Mortality, body weight, ophthalmology, cardiovascular, bone, physical and neurological exams, neurobehavioral observations, learning and memory, clinical pathology, histopathology.
  - TK
  - Low percentage of ADA
  - Deficit in lower spinal reflexes in HD, neurobehavioral (learning and memory) deficit in MD: NOAEL: 0.3 mg
- Genetic toxicology: Negative in Ames, in vitro chromosome aberration, in vitro micronucleus assays.
- Combined fertility and developmental toxicity study via subcutaneous injection in CD mice: 0, 3, 10, 25 mg/kg SC every other day prior to and during mating and pregnancy: No clear effect on embryofetal development.
- Embryo-fetal developmental toxicity:in rabbit: 6, 12.6, 25 mg/kg: No clear evidence of maternal or embryofetal toxicity.
- Other tox studies: hippocampal vacuolation in monkeys

- **Conclusion**

Comparison of Biologics (case by case) vs Small Molecules (more standardized)

	Small molecules	Biologics
MW	~ 200-500	~ 150 KDa (typically)
Test article	Chemical	Protein
Physicochemical properties	Mostly well-defined physicochemical properties	Complex physicochemical properties (e.g. tertiary structure, stability, PTM)
ADME tools	Available/ extensive ADME understanding	Understanding of ADME still evolving
Dosing route	Oral often possible	Usually parenterally (IV, SC, and IM), Intravitreal injection
Dose interval	Daily (typically)	Intermittent dosing
Half-life ($t_{1/2}$)	Short (typically several to 24 hrs)	Long (typically days or weeks)

Source: Hong Wan. *ADMET & DMPK* 4(1) (2016) 1-22

Comparison of Biologics vs Small Molecules (cont.)

	Small molecules	Biologics
Distribution (V_d)	High V_d, distribution to organs/tissues Potential substrate of transporters	Lower V_d, usually limited to plasma and/or extracellular fluids
Metabolism pathway	Mainly by CYP enzymes and phase II enzymes, metabolized to non-active and active metabolites	Catabolism Degraded to peptides or amino acids
Drug metabolite safety evaluation	Yes	No
Excretion	Mainly biliary and renal excretion	Mostly recycled by body

Comparison of Biologics vs Small Molecules (cont.)

	Small molecules	Biologics
Clearance (CL)	Mostly linear PK; non-linearity mainly due to saturation of metabolic pathways	Slow clearance
Potency and selectivity	Generally less selective	High selectivity (affinity/ potency)
PK analytes	Drug and metabolites	Antibody and ADA
PK bioanalysis	LC-MS/MS methods	Mostly ELISA (total antibody), Recently with increased LC-MS/MS applications
PD	Short acting	Long acting

Comparison of Biologics vs Small Molecules (cont.)

	Small molecules	Biologics
PK/PD	PK usually not driven by PD due to dominance of non-target mediated binding	PK and PD mechanistically connected (TMDD)
DDI	Many examples and PK and/or PD related (by CYP enzymes or transporters)	Sparse examples and mostly PD related
hERG	Yes	No
Immunogenicity	No	Yes
Toxicity	On-and off-target related toxicity	Typically exaggerated pharmacology
Formulation	Complex and diverse	Simple formulation
API/Production Process	Synthesized (uniform single entity) Generics, bioequivalence	Culture-derived (generally nonuniform) No generics, biosimilar or comparability

Thanks for Listening!