Non-clinical approaches for immunogenicity assessment: predictive models

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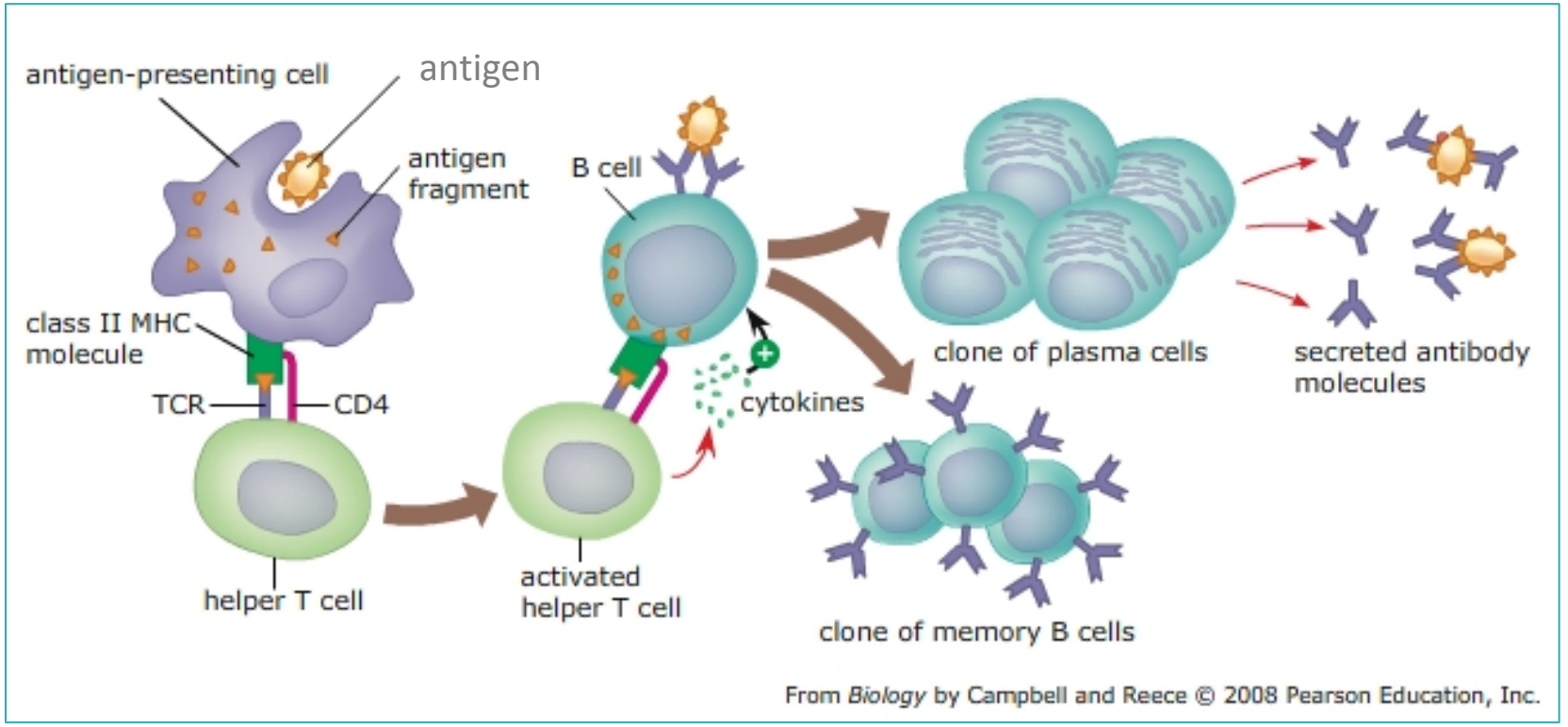
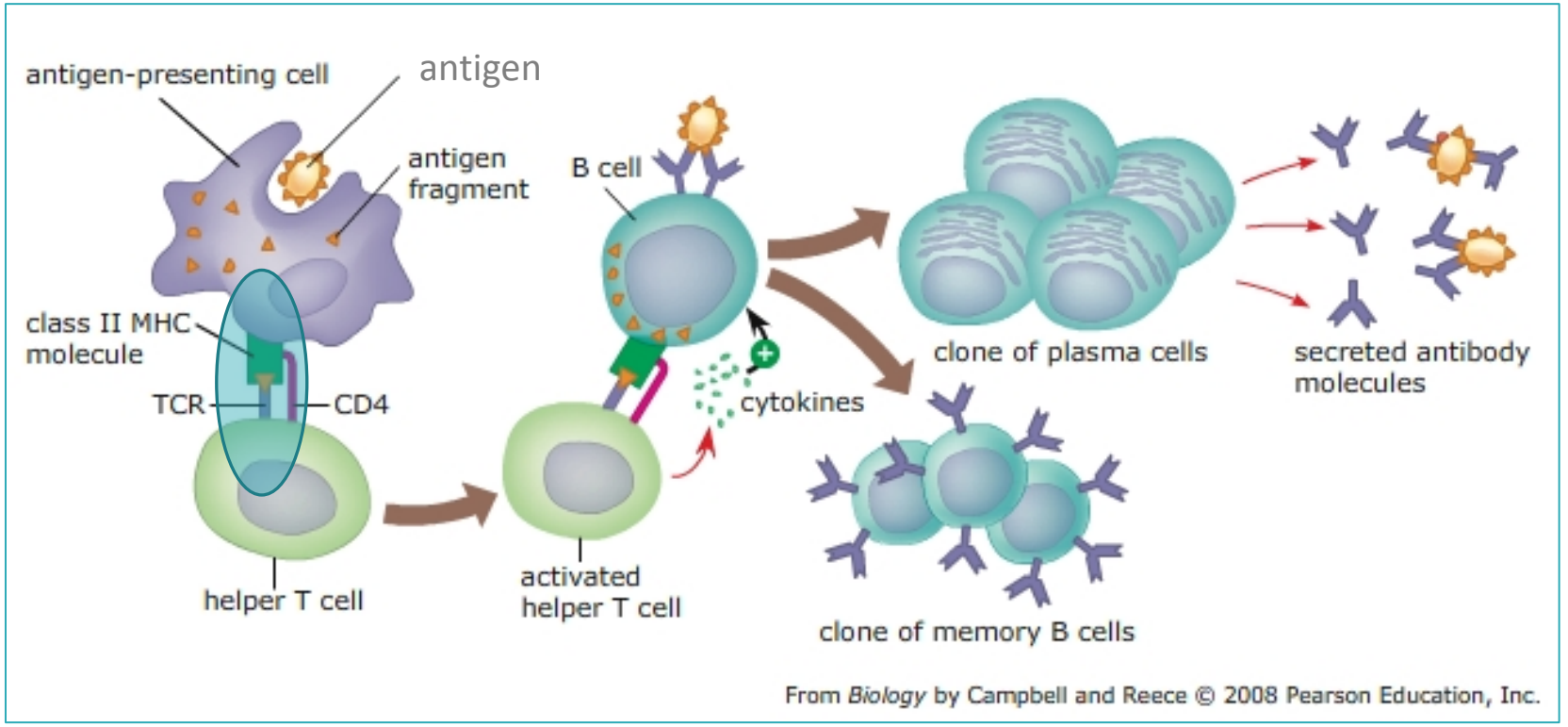
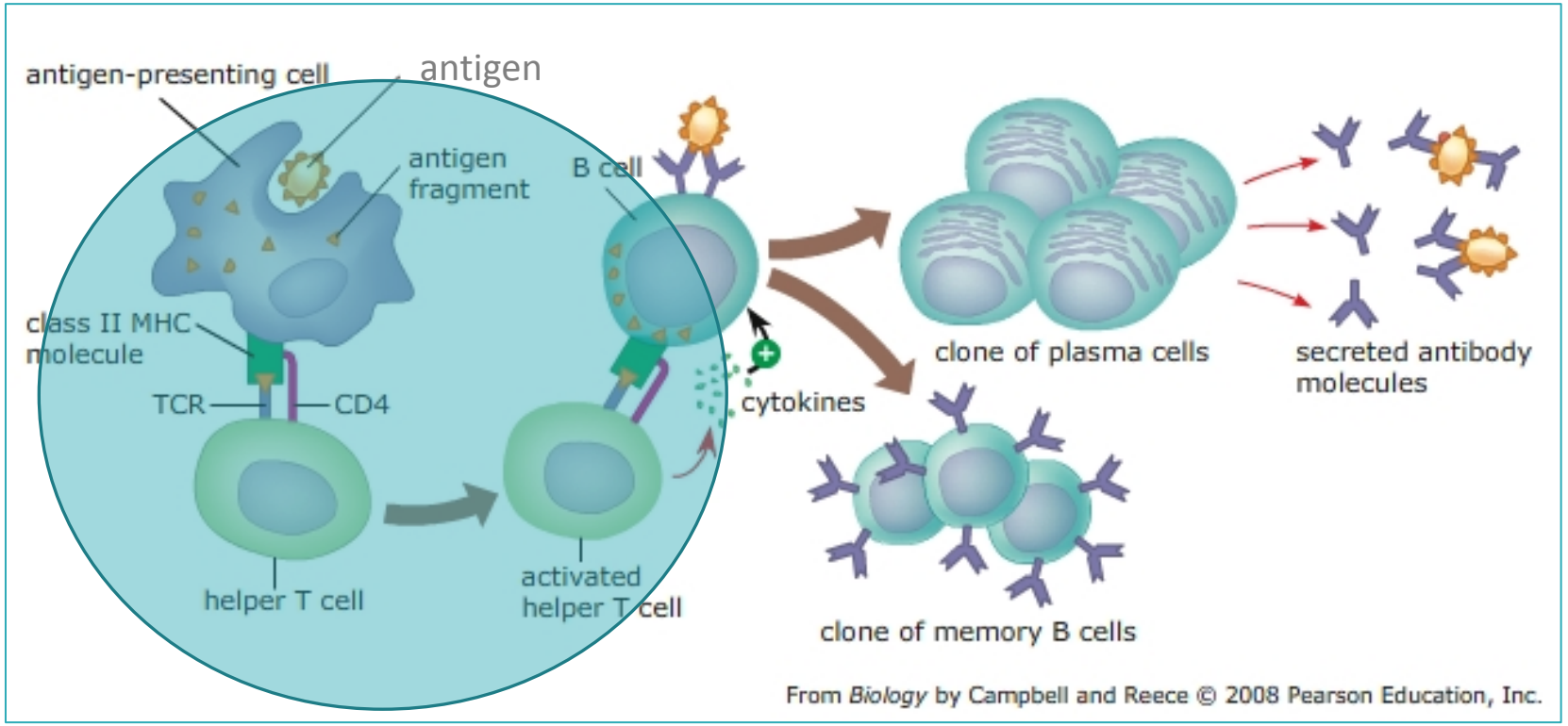
Immunogenicity Assessment



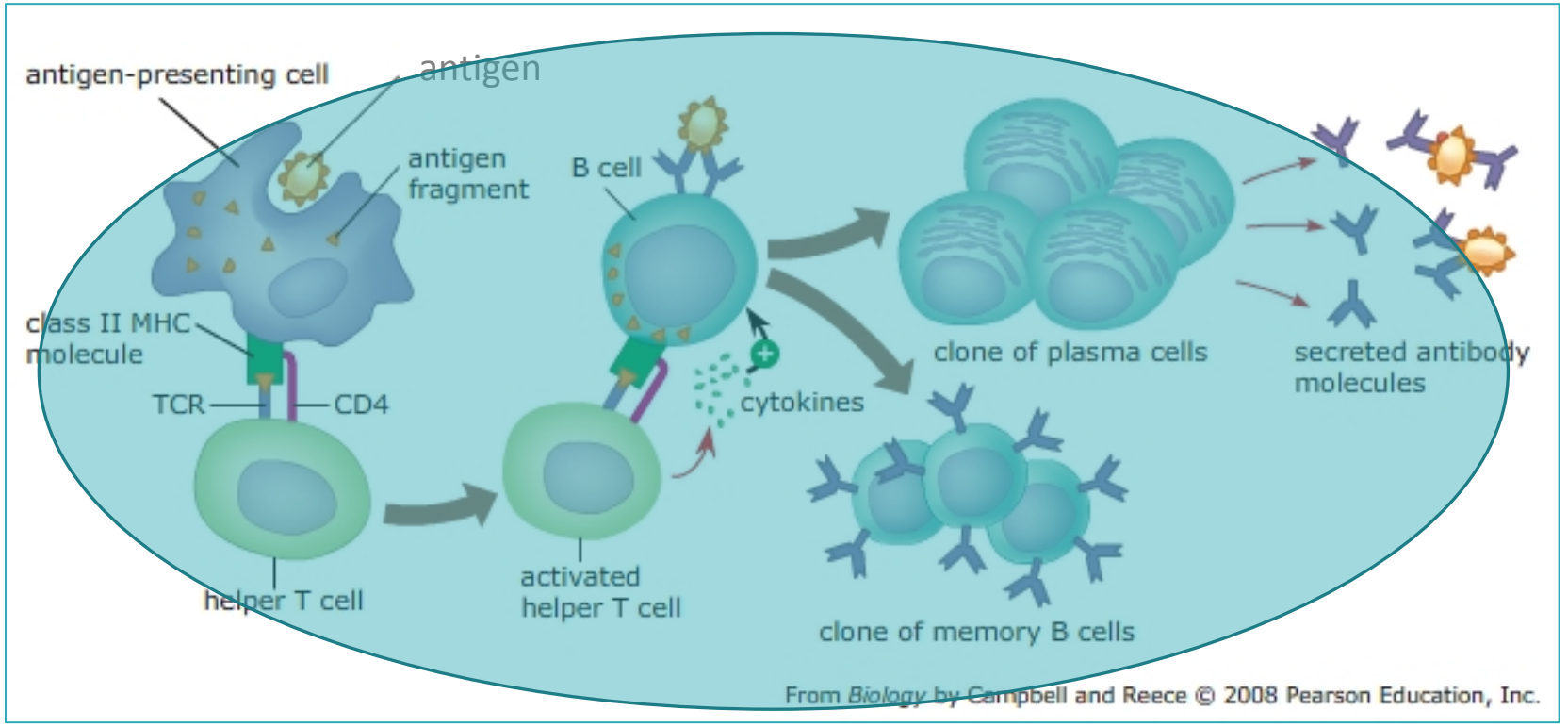
In silico tools



In vitro tools



In vivo tools



In vivo tools: challenges

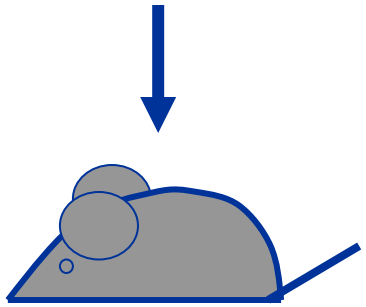
- Human proteins administered to animals will be recognized as foreign proteins.
- Mechanism of antibody induction depends on the origin and the immunological characteristics of the product.
  - classic immune response vs. breaking immune tolerance
- Humans and animals have different immune systems.
  - e.g. diverse MHC genes between species
- Choice of appropriate reference product for comparative immunogenicity assessment is crucial.

In vivo tools: models

- **Conventional Animal models**
  - Check whether ADA interfere with PK/PD studies
  - Measure ADAs to help interpretation potential findings in Toxicity studies
- **Mice rendered immune tolerant to human therapeutic proteins (transgenic, knock in)**
  - Breaking tolerance
  - Drug specific (e.g. IFN, FVIII)
- **HLA transgenic mice**
  - Discovery of T-cell epitopes for a specific haplotype
- **Human immune system xenograft models**
  - immunodeficient NOD scid IL2Rγ-/- or Rag2-/-γc-/- mice
  - Full human immune system
  - Very expensive to cover world population (# of grafted mice)
  - Cannot be made genetically

In vivo methods: shortcomings

human protein drug

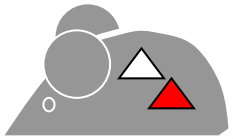


conventional mouse model

antibody responses to xenogeneic human proteins that are recognized as foreign → **knockout mouse protein and introduce human protein of interest as a transgen**

presentation of immunogenic peptides by murine antigen-presenting complex (MHC-class II) that drives $CD4^+$ T cell responses required for the development of high-affinity antibodies → **exchange murine against human MHC-class II**

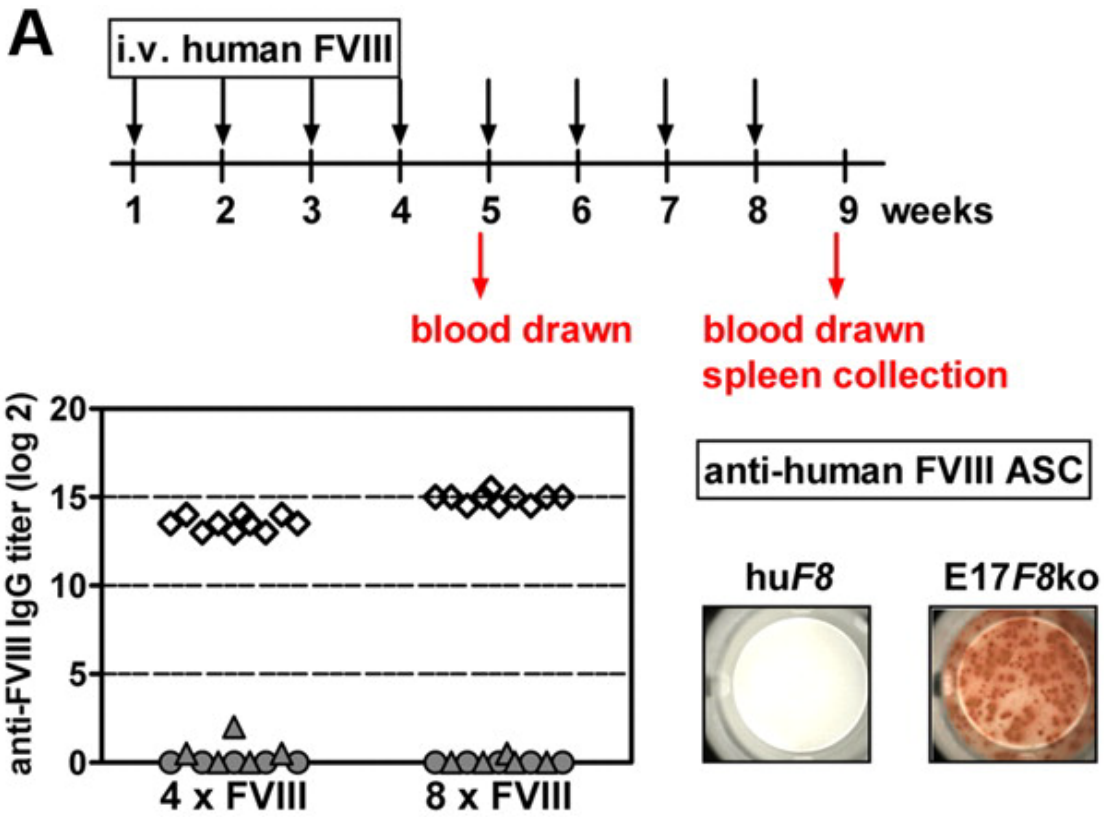
Human F8 transgenic hemophilic mouse model



van Helden PM et al.: Blood 2011;118(13):3698-370

- knockout of murine factor VIII (E17, △) to generate mice with hemophilia A
- random integration of human *factor 8* transgen (▲), albumin promoter directs expression to the liver
- Specific immune tolerance to human factor VIII, antibody response to unrelated human proteins is normal
- Develop antibodies against human FVIII only if the immune tolerance breaks down
- This animal model reflects the situation in previously treated hemophilia A patients without antibodies against FVIII

Human F8 transgenic hemophilic mouse model



van Helden et al, Blood: 118 (13), 2011

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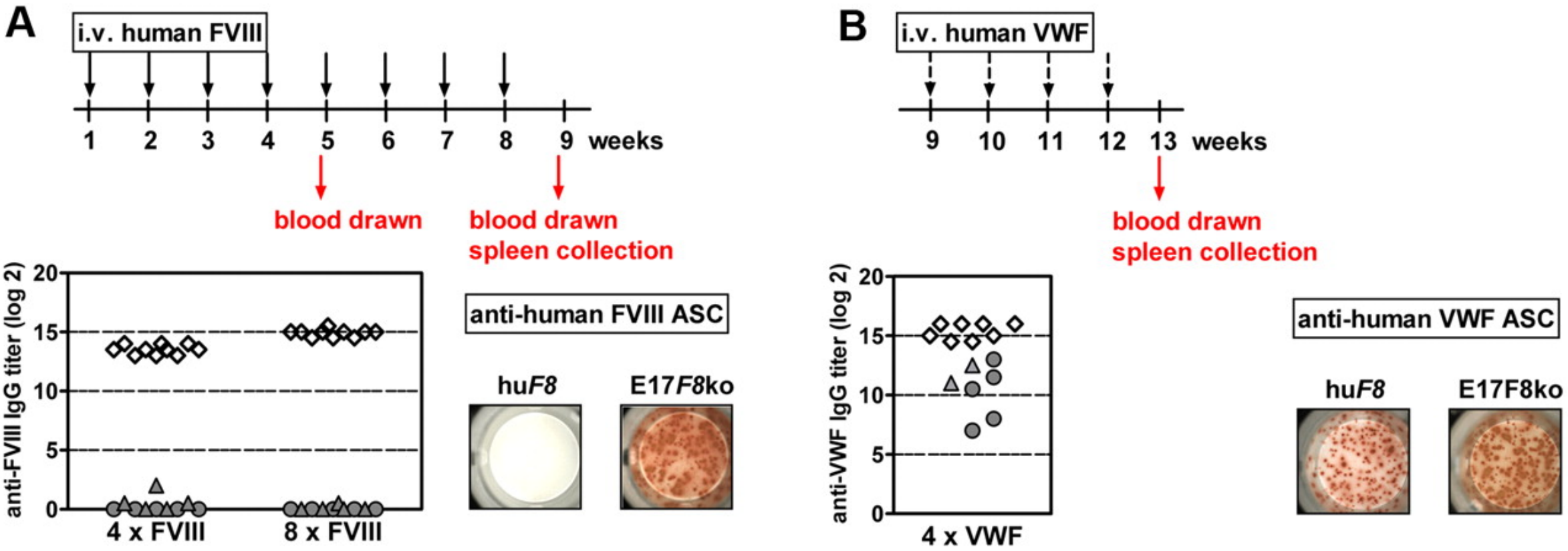

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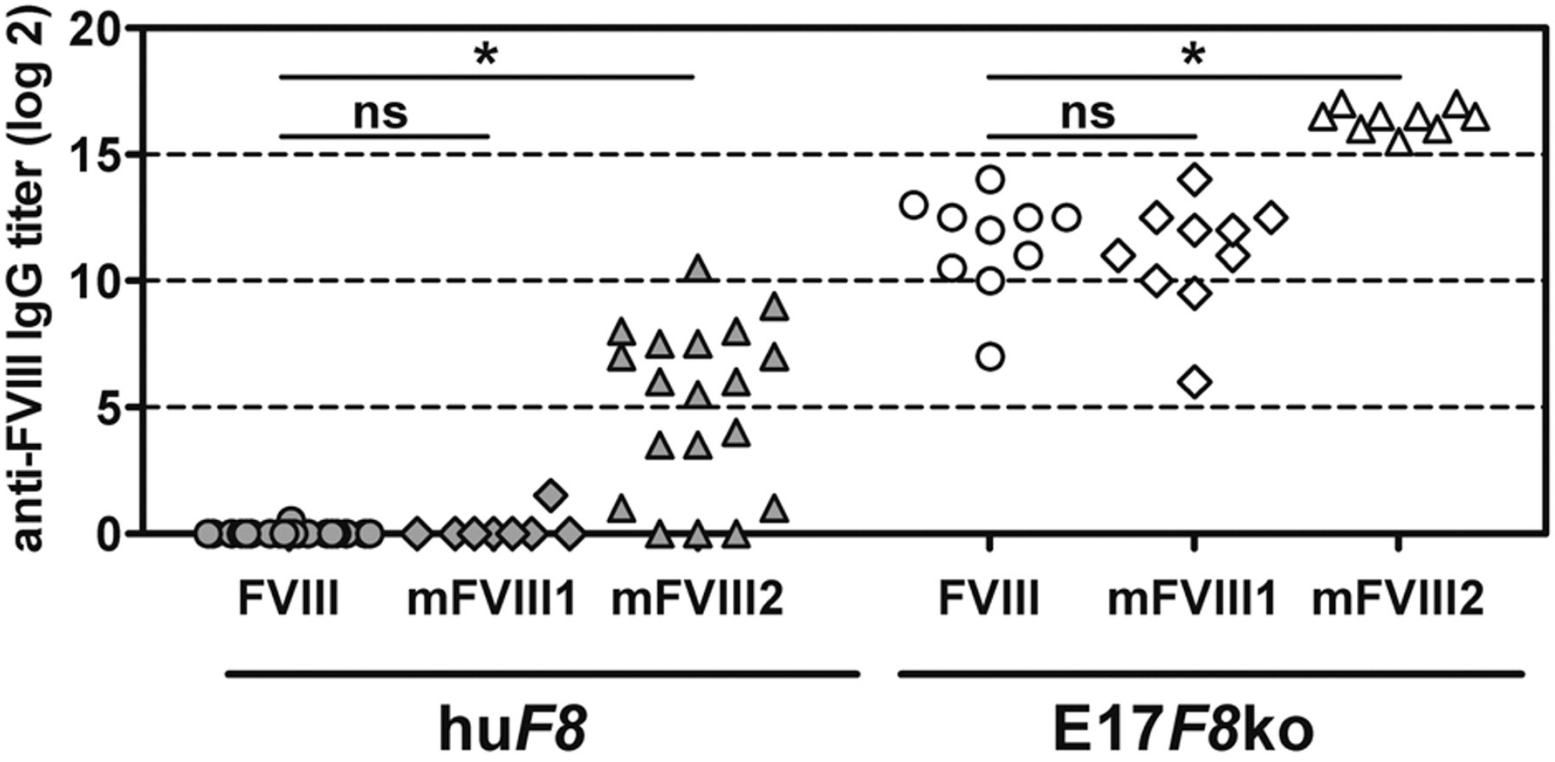

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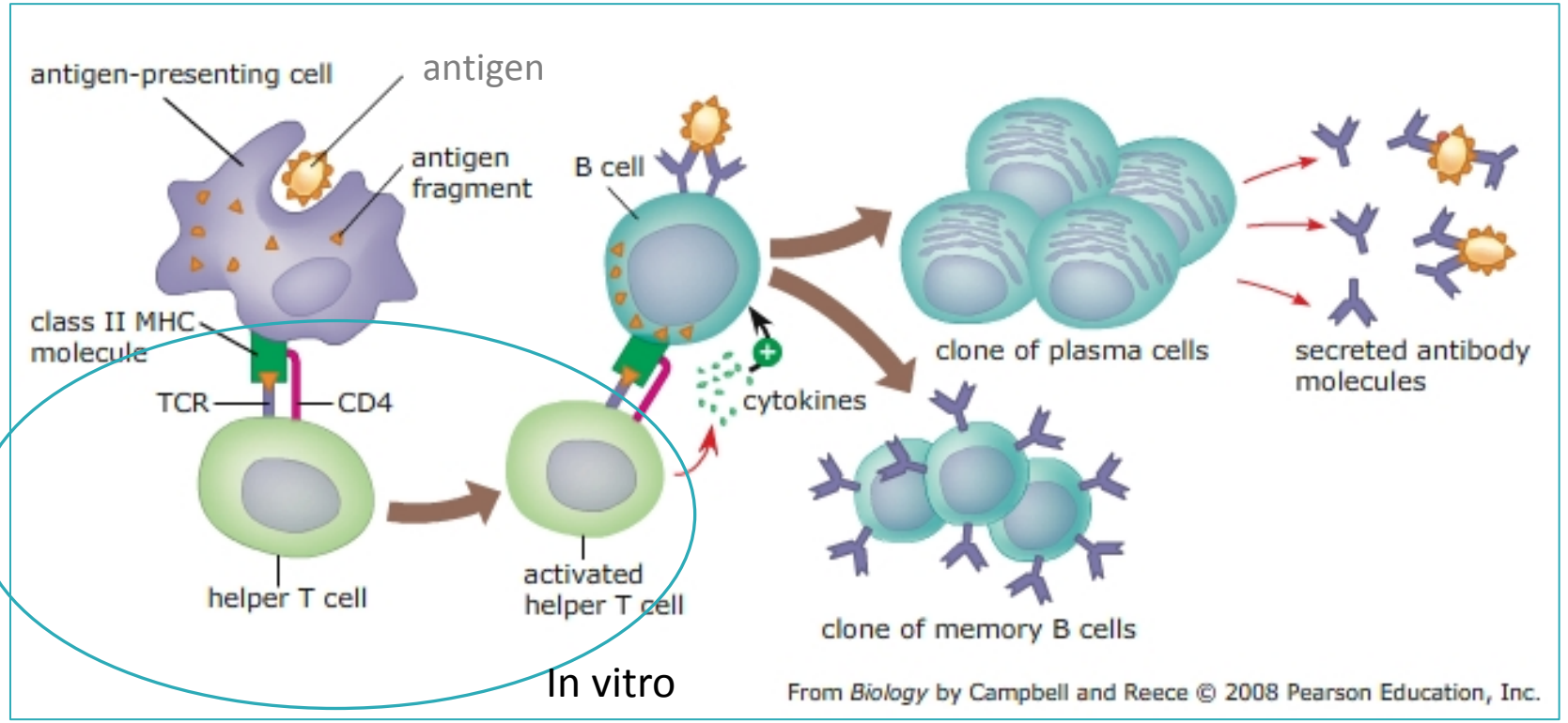
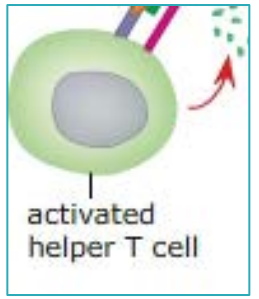
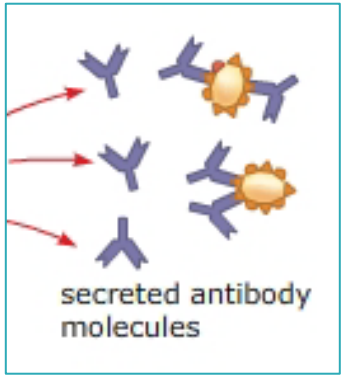
In vitro methods



In vitro methods

- ✓ HLA binding peptide discovery:
  - ✓ MAPP: Epitope Elution from APC
    - ✓ Guiding other in vitro and in vivo studies
  - ✓ Peptide-HLA binding testing
    - ✓ Alternative to in silico tools
- ✓ T cell activation/proliferation studies
  - ✓ Used as a surrogate marker for antibody responses
  - ✓ PBMC or DC assays
  - ✓ Whole protein (overall risk) or peptide (T cell epitope identification)
- ✓ B-cell activation studies
  - ✓ Novel technologies mapping naive B-cell responses
- ✓ DC uptake and activation assays: innate immunity vs humoral response
- ✓ Human In vitro cytokine release assay and surface marker analysis to map innate immune responses





In vitro tools

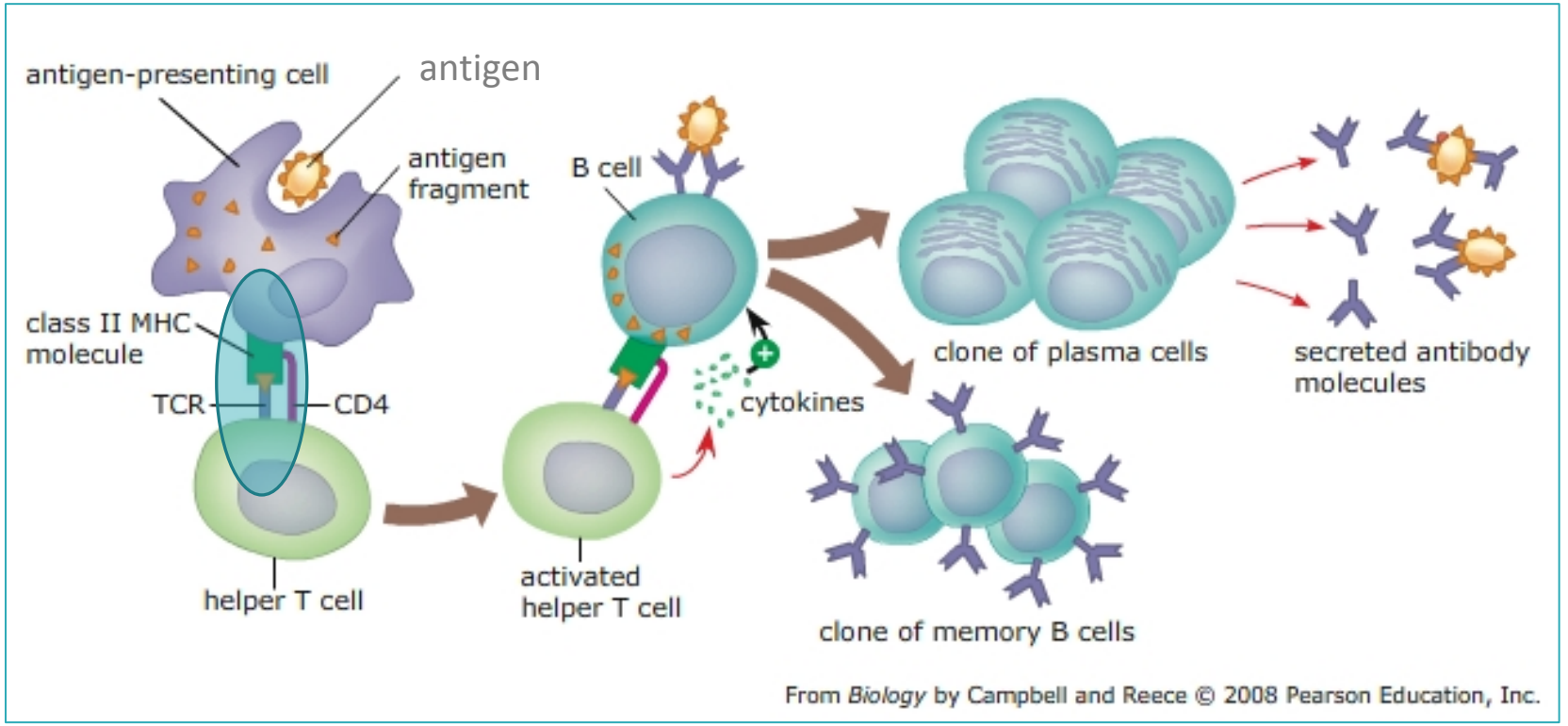
Uses for Immunogenicity Assessment

- Comparing different drug candidates
- Use in risk management plan or in design of clinical trials
- Characterize drug candidates

- Takes into account drug in formulation
- Takes into account aggregates, HCP, Post-translational-modifications, …
- Allows to compare drugs candidates with similar or equal sequence (e.g. Biosimilars)

- Requires large amounts of cell material
- Sufficient donors to be included to ensure representative sample of world-population (HLA coverage)

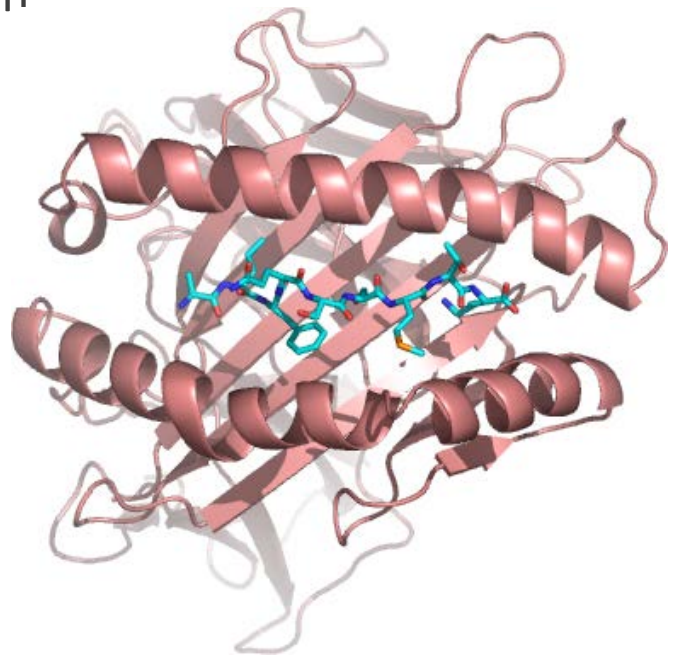
In silico tools



In silico tools

Peptide-HLA Binding Identification

- ✓ Putative T-cell epitopes
- ✓ Statistical and structure-based methods or a combination of both
- ✓ Study of peptide-HLA interactions
- ✓ Assessment of impact on specific populations
- ✓ Relative Immungonenicity assessment (not absolute)
- ✓ Examples: NetMHCIIpan, PreDeFT, Epibase™, iTope™, ….



In silico tools

T-cell epitope mapping

- Methods are cheap, easy to use and by definition overpredictive
  - Binding to HLA not a sufficient step to be epitope
  - No differentiation between T-cell subtypes that will be activated
  - Self-peptides are also binders
  - Some peptides are never presented as they are cleaved in the endosomes
- Methods do not take into account other signals contributing to immunogenicity (aggregates, post translational modifications, HCP, ...)
- Methods require a broad HLA coverage to be representative for the human population

Uses for Immunogenicity Assessment

- Comparing different drug candidates
- Guide protein engineering studies (e.g. humanization, directed evolution, library design and deimmunization)
- Use in risk management plan or in design of clinical trials
- Characterize drug candidates

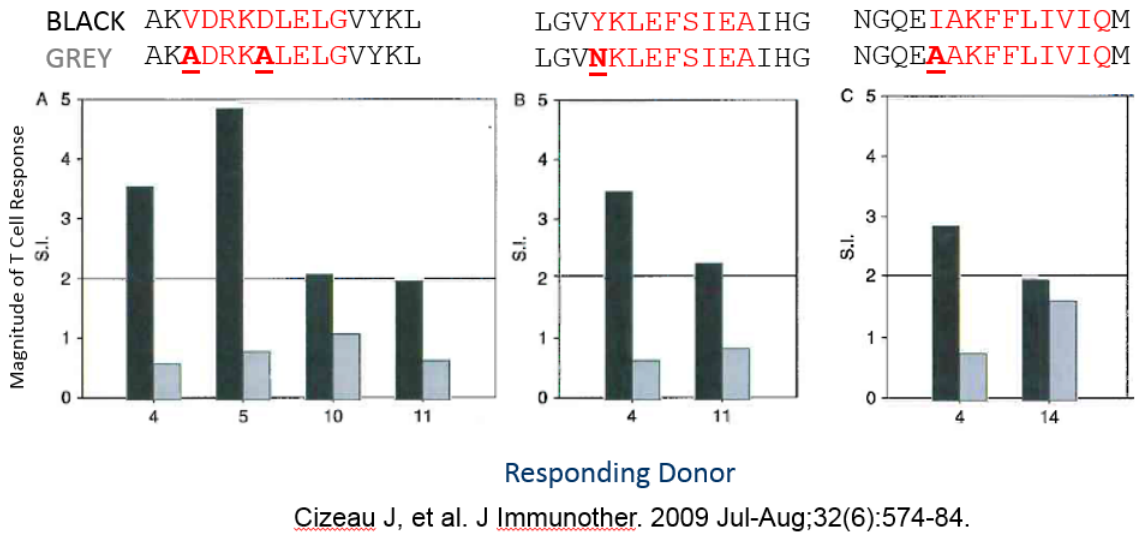
Case study

- Viventia's anti-EpCAM V6-845 recombinant immunotoxin
  - Humanized Fab fragment fused to a deimmunized toxin (bouganin)
- Targets and mediates cell death in EpCAM-positive solid tumors
- Phase I trial assessed the safety of VB6-845 in 13 patients with various EpCAM-positive cancers (2008)
  - Low or no antibody responses against deimmunized bouganin portion
  - Observed immune response to Fab moiety

◦ Subsequent de-immunization of the Fab Moiety
  ◦ In silico deimmunization
  ◦ In vitro verification (T-cell reponses in PBMC of healthy donors)

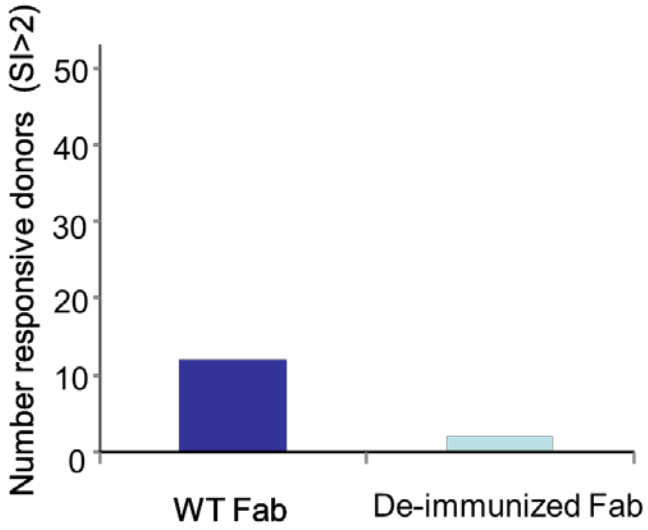
◦ Viventia earlier-stage programs are focused on de-immunized, systemically-administered product candidates, including VB6-845d, being developed for the treatment of multiple types of EpCAM-positive solid tumors and expected to enter a Phase 1/2 clinical trial in the first quarter of 2016

Case study



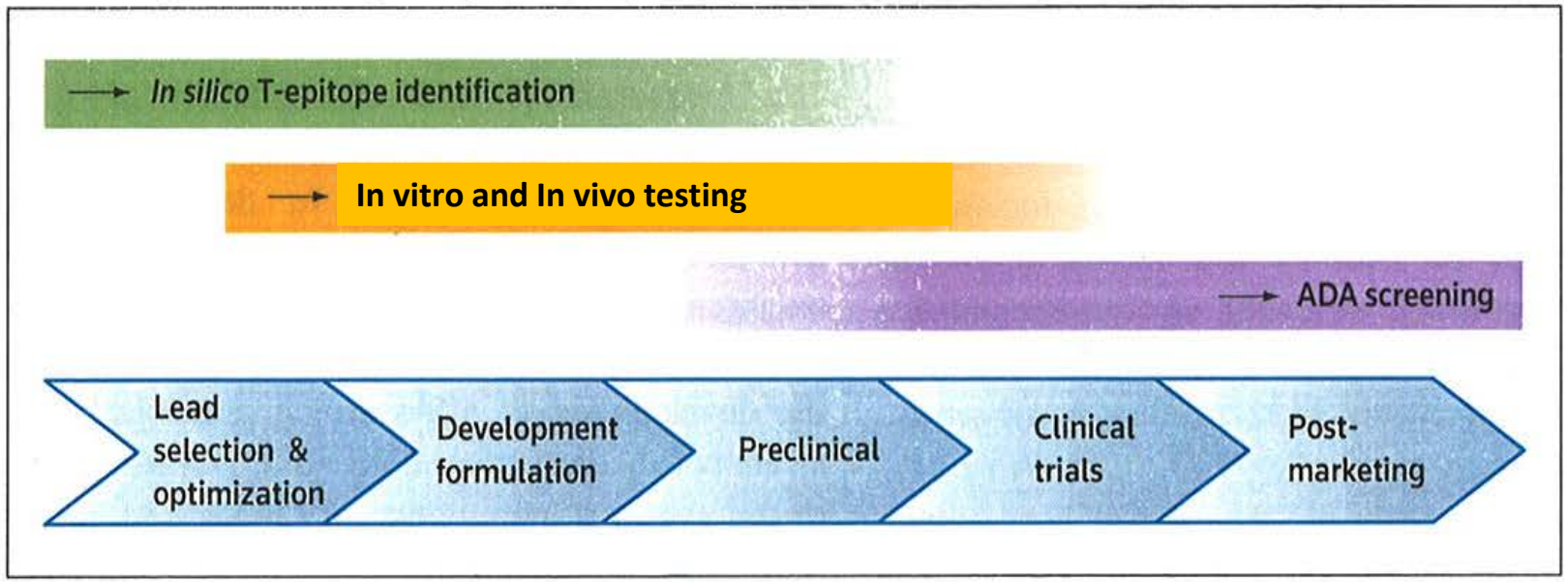
Cizeau J, et al. J Immunother. 2009 Jul-Aug;32(6):574-84.

T-cell activation study on bouganin and deBouganin peptides



T-cell activation study (50 donors) on WT anti-EpCAM-Fab and deimmunized version

Immunogenicity Assessment tools



For discussion

- Is a preclinical immunogenicity risk assessment mandatory for CTA?
- Strategy for preclinical immunogenicity is a case by case decision
  - Type of compound (antibody, enzyme, peptide, biosimilar)
  - Modification of compound (hybrid molecules, chemically modified)
  - Availability or relevant reference product
- Aim of the immunogenicity risk assessment
  - Interpretation of preclinical PK/PD and Toxicity studies
  - Lead candidate selection
  - Evaluation of antibody response in humans