BIOTEM

custom antibodies & services

Ultimate Humanization™ Platform

Towards the 4th Generation of Therapeutic Recombinant Antibodies?

Our Commitments make the Difference!

BIOTEM: Company Presentation

- Contract Research Organization (C.R.O.) in **immunotechnology** since **1980**
- High qualified staff (30 employees including 7 PhD and 7 engineers)
- 2000 m^2 facility (Apprieu - Rhône-Alpes)
- ISO 9001 + CIR certifications

BIOTEM: Activity Overview & Applications

Therapeutic Antibody

Immunoassay Development

Rabbit Monoclonal Antibody

Mouse & Rat Monoclonal Antibody

Polyclonal Antibody

Ultimate Humanization™
Therapeutic Antibodies
4th Generation

Fully Human Antibodies
EBV-immortalization

Humanized Antibodies
CDR-grafting

ELISA & Lateral Flow
Assay Development
Validation
Production

Chimeric Antibodies

THERAPEUTICS

DIAGNOSTICS

R & D

Therapeutic antibodies: Are the latest generations fully satisfactory?

Murine Antibodies

1st Generation Chimeric Antibodies

2nd Generation Humanized Antibodies* (CDR grafting)

3rd Generation "Fully Human" Antibodies*

*Most commercialized therapeutic antibodies to date

Proportion of human sequences

- Overall **success rate** from pre-clinical studies to market phase: <10%
  - Immunogenicity
  - Efficacy
  - Toxicity : Cross-reactivity ("*off target interactions*")
  - Other factors...

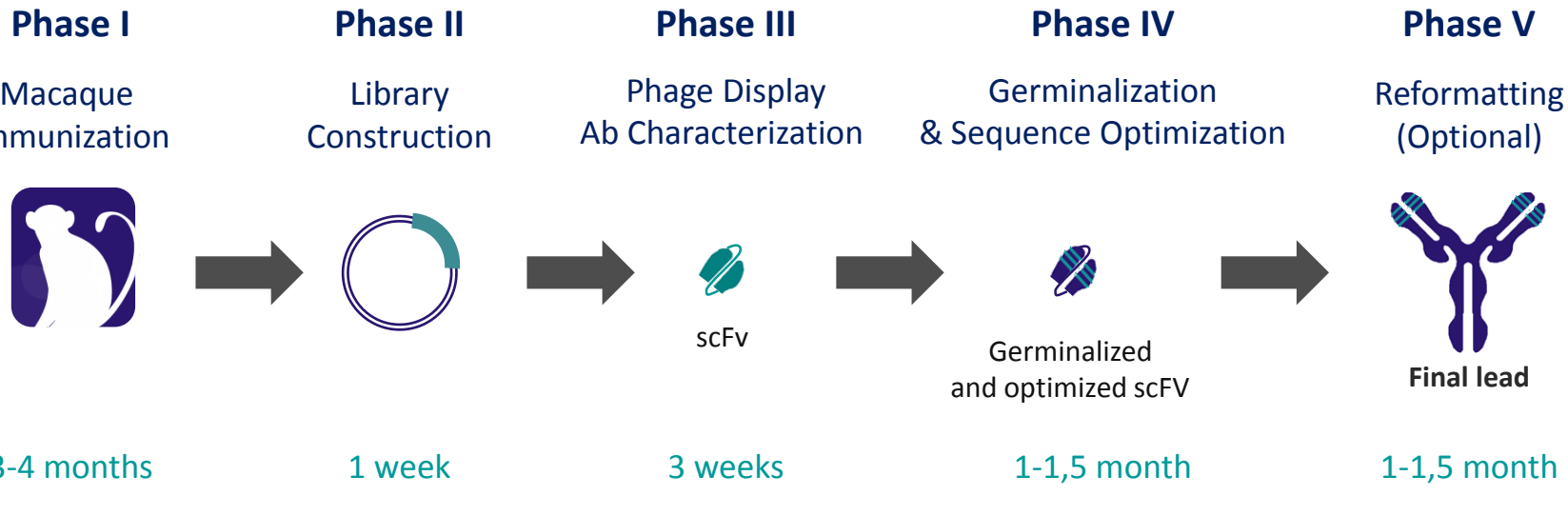
Ab with side effects

Therapeutic antibodies: What should be the next 4th generation ?

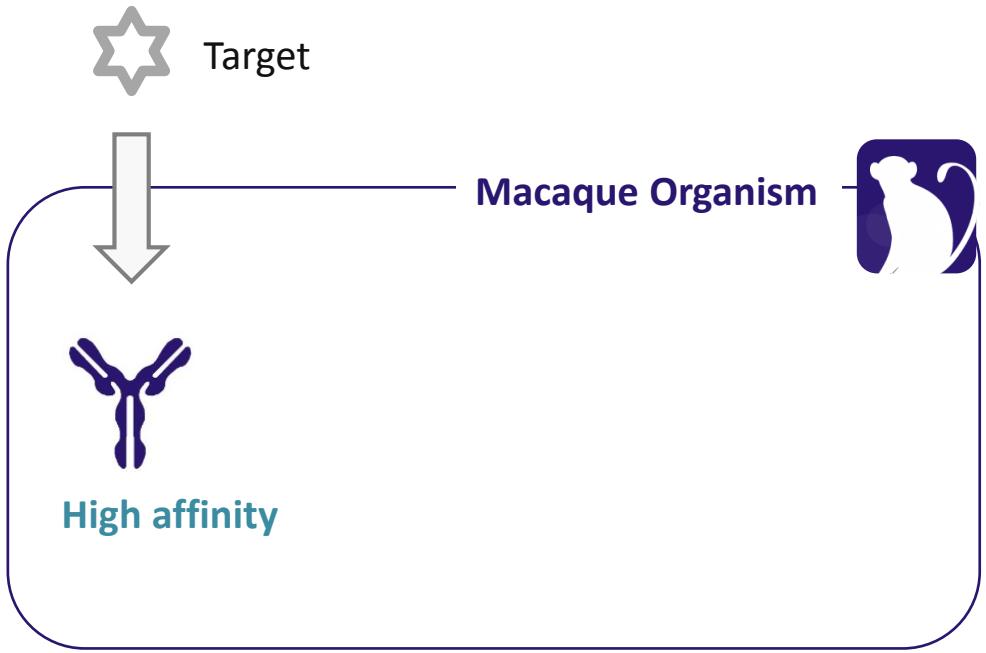
Ultimate Humanization™ Platform

- BIOTEM's strategy to optimize **success rate**
- Recombinant antibodies derived from active immunization of macaques

Technology Overview



Advantage #1: High affinity antibodies



- Active immunization allows the generation of **high affinity antibodies** against virtually **any type of target** (virus, bacteria, human proteins,…)

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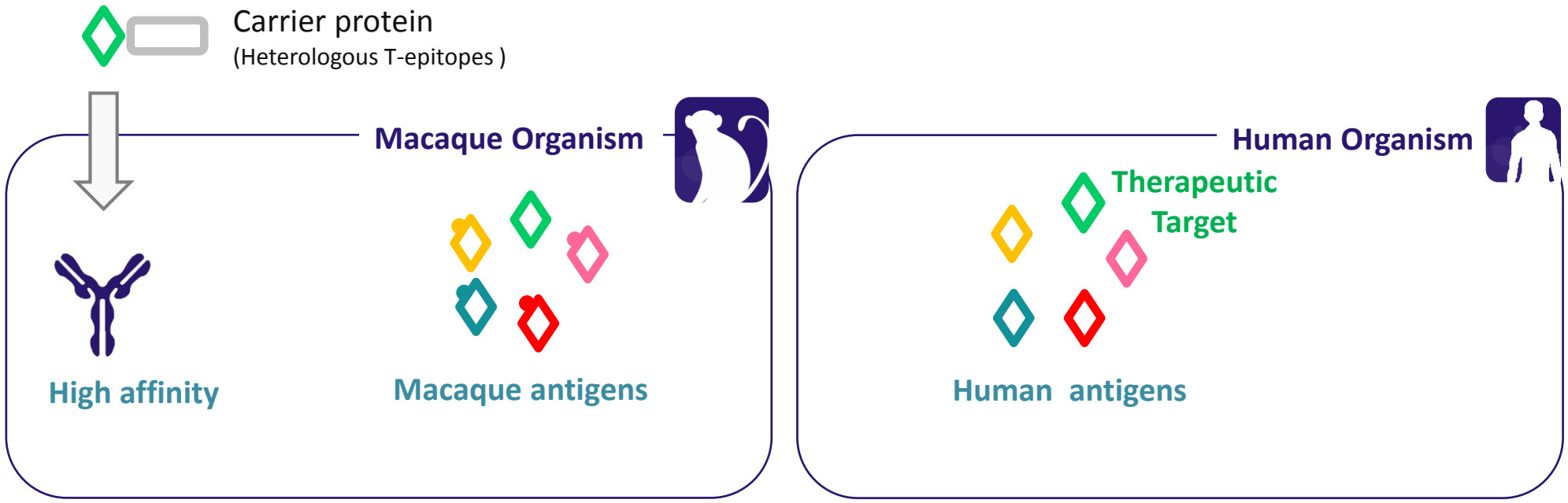
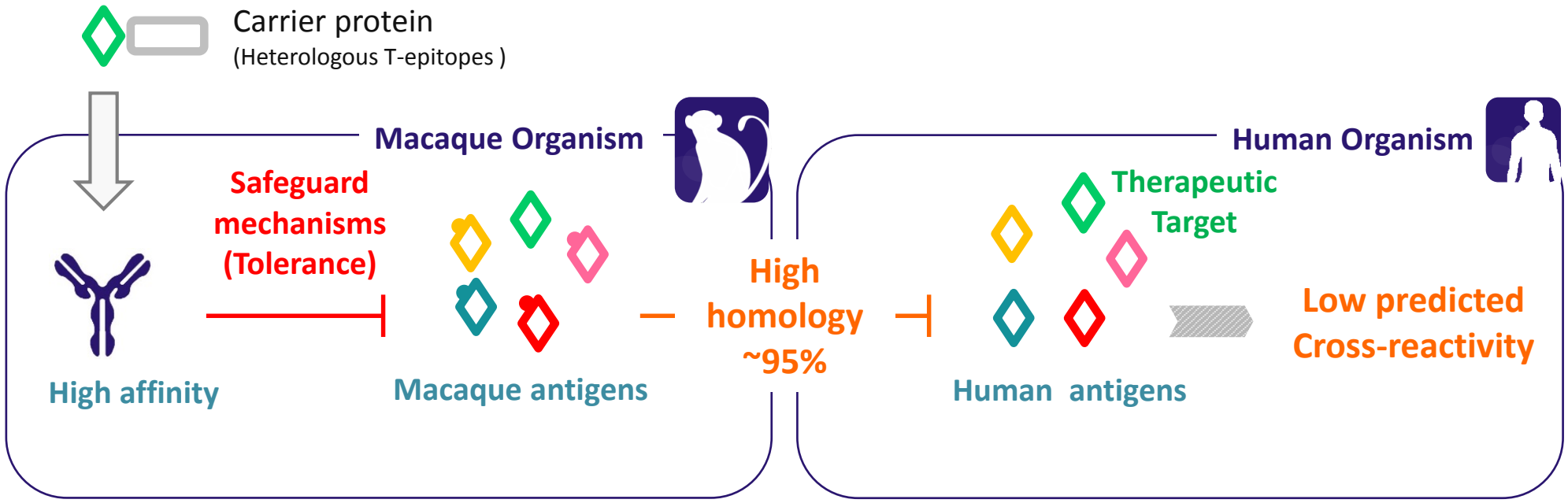


- Active immunization allows the generation of **high affinity antibodies** against virtually **any type of target** (virus, bacteria, human proteins,…)

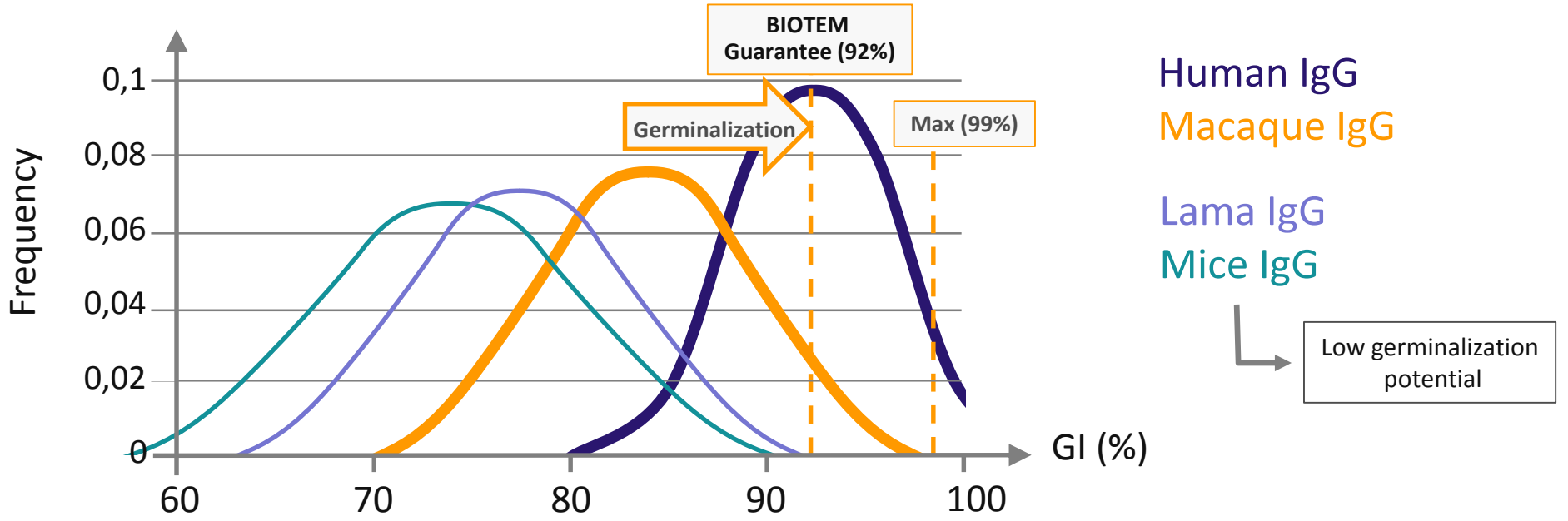
Highly conserved proteins: Immunizations are performed with conjugates containing heterologous T-cell epitopes to avoid immune tolerance

Advantage #2: Low predicted cross-reactive antibodies



- Macaque immunization should produce antibodies with **low predicted cross-reactivity** against human antigens ("off target interactions")
- Minimize **toxicity risks** at **early stage** of development

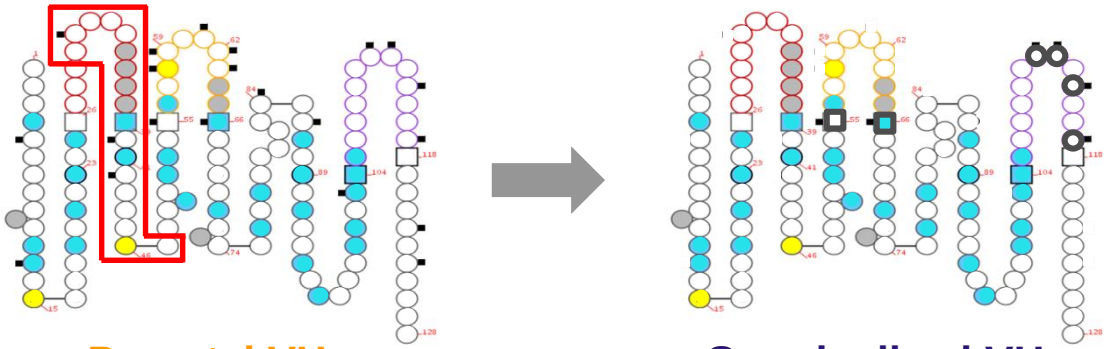
Advantage #3: Extensive germinalization



- Macaques naturally produce antibodies with high homology to human germline V-regions (quantified by the **Germinality Index**)
- This unique property allows **extensive germinalization**: Mutations in FR and CDR regions to increase the GI (without altering antibody affinity and specificity)
- Minimize **immunogenicity risks** (and potentiate efficiency)

Case study: Germinalization of a macaque monoclonal antibody

1 T cell epitope

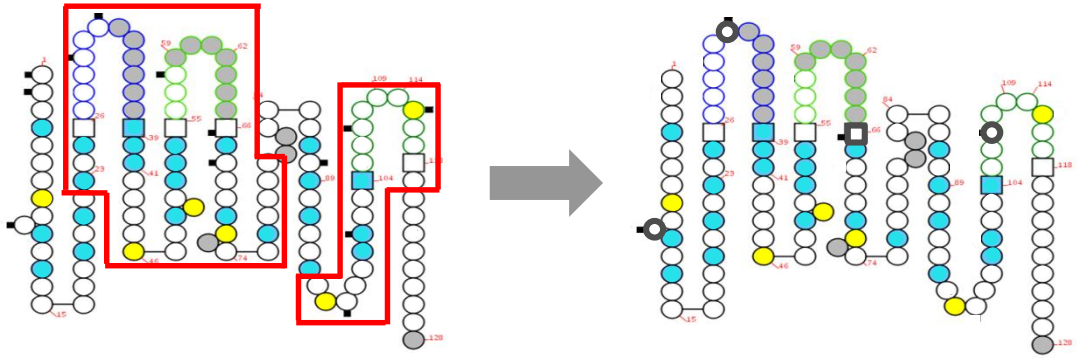


Parental VH

Germinalized VH

GI	85,0 %	95,6 %

3 T cell epitopes

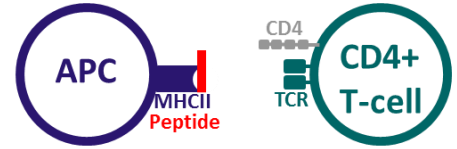


Parental VL

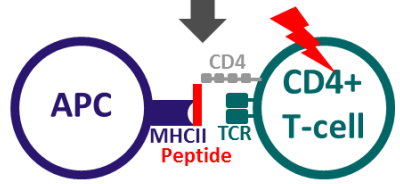
Germinalized VL

- **Germinalization** (FR + CDR)
- **100%** target affinity preserved
- Efficient removal of all T cell epitopes in the germinalized candidate

T cell epitopes methodology



Binding of peptides to MHCII



T-cell activation (proliferation)

Ultimate Humanization™ Platform : Comparison with previous generations…

Technology		Generation	Affinity	GI (V region)	ADA Risks (Immunogenicity)	Toxicity Risks (off target)	Comments
Ultimate Humanization™		4	High	High > 92%	Low	Low Probability	No Claims No royalties No follow-up rights
Fully Human Antibodies	**Transgenic Mice**	3	High	High	Low	**Possible**	**Strong IP (Licensing)**
	Human B-cell cloning (EBV, single cell strategies)					Low Probability	**Targets & donnors restricted technologies**
	Phage Display (Human immune libraries)						
	Phage Display (Human naïve libraries)		High	**?**	**?**	**Possible**	**Affinity maturation required**
			Low	High	Low		
Humanized Antibody (Rodent CDR grafting)		2	**Low**	High	Low	**Possible**	**Balance between affinity and germinality Index**
			High	**Medium**	**Medium**		
Chimeric Antibodies (Human / Rodent)		1	High	**Low**	**High**	**Possible**	
Rodent Antibodies			High	**Low**	**Very High**	**Possible**	

- The Ultimate Humanization™ Platform offers significant advantages over the 2nd generation and some of the 3rd generation technologies

Technology available at BIOTEM

Thank you for your attention

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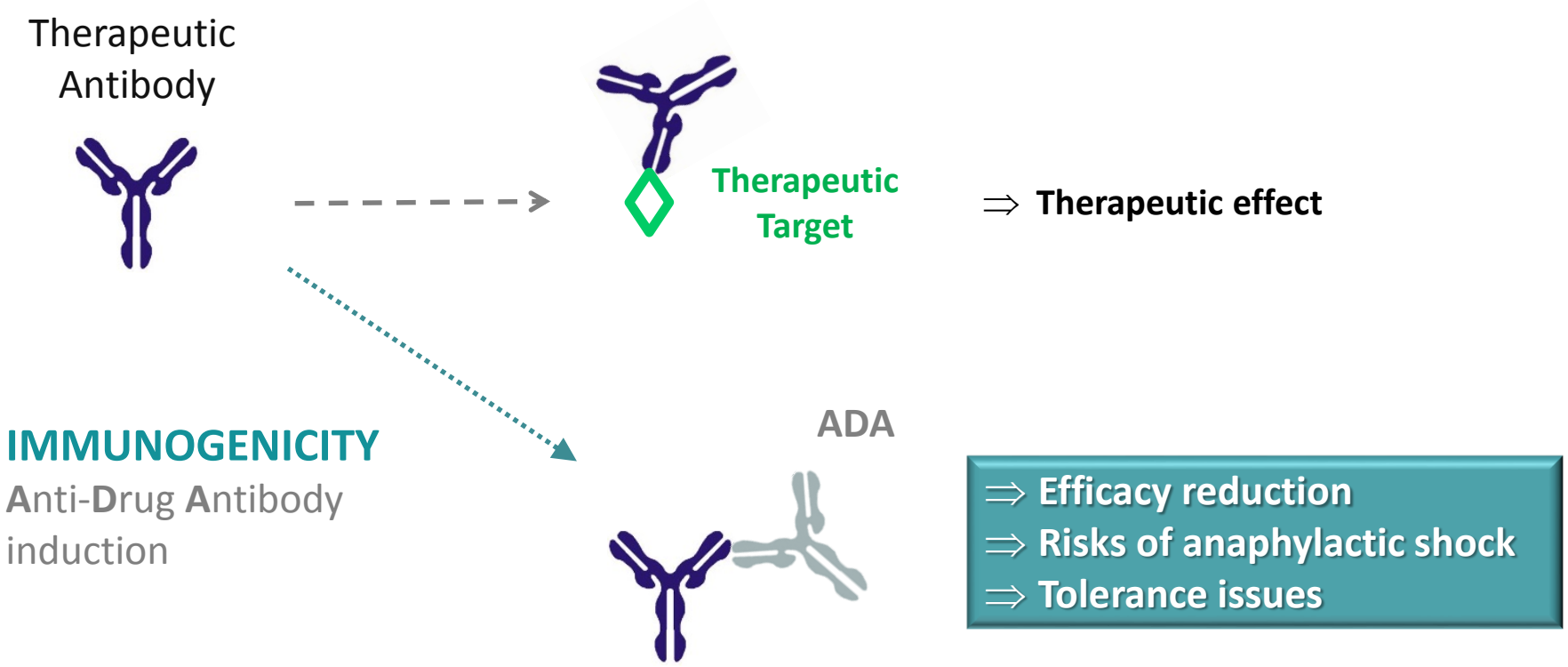
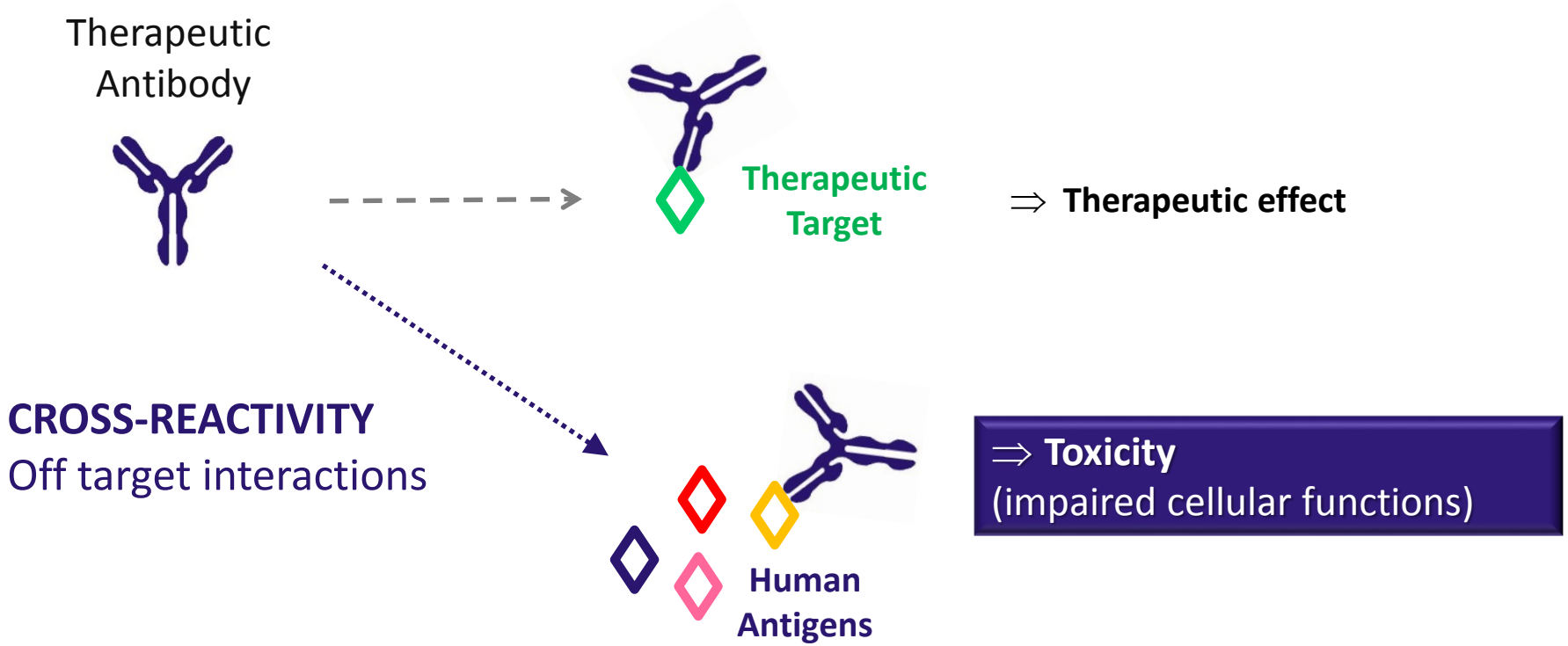
Risks to consider during antibody development: Immunogenicity



- **Anti-Drug Antibodies** (ADA) may reduce both **efficacy** and **safety**
- Many factors described to date leading to a multitude of sequence optimization strategies

Risks to consider during antibody development: Cross-reactivity



- Cross-reactive antibodies may impaired important cellular functions and cause **toxicity**
- Most available strategies for antibody development do not address at all this crucial issue

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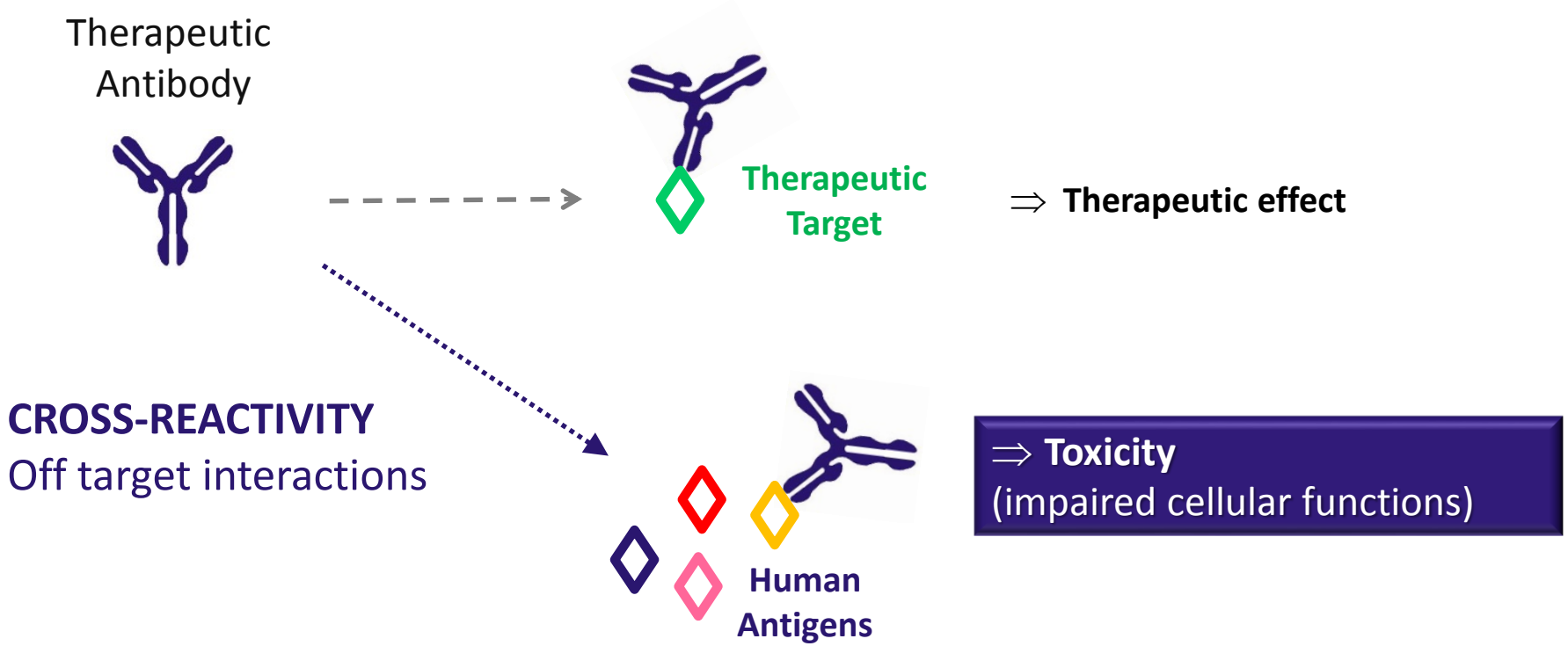

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Therapeutic antibodies: Factors impacting immunogenicity

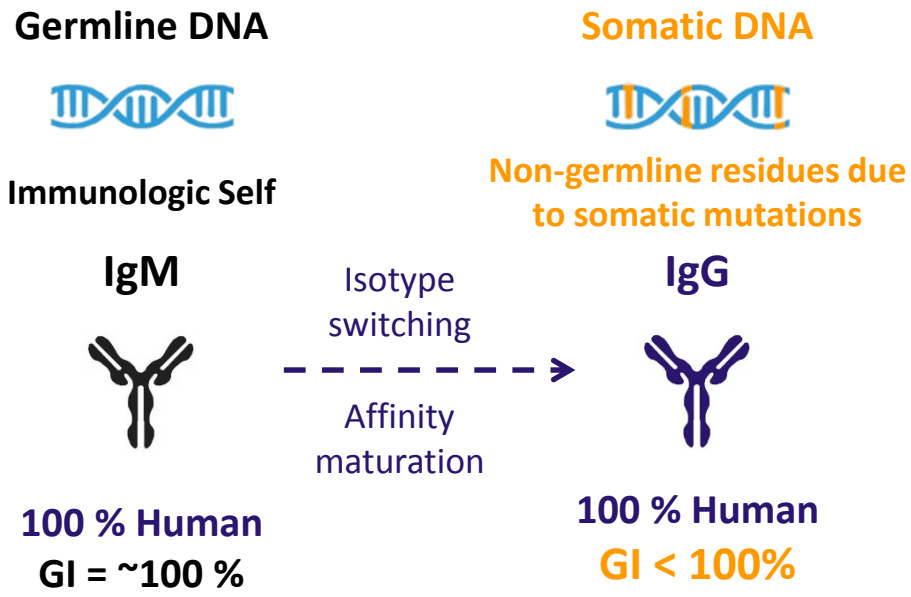
- **“Humanness degree”** (Best evaluation using the **Germinality Index**)
- Post translation modifications (unusual glycosylation,…)
- Denaturation (deamidation, oxidation,…)*
- Formation of aggregates*
- Human Ig allotypes
- Method and frequency of administrations
- Antibody dosage
- Patients’ disease and/or immune status
- Patients’ MHC haplotype
- Cell-surface or soluble antigen?
- IC Formation with antigen
- Complement activation by antibody
- Fc receptor binding by antibody
- Inflammation and cytokine release

Not related to antibody sequence

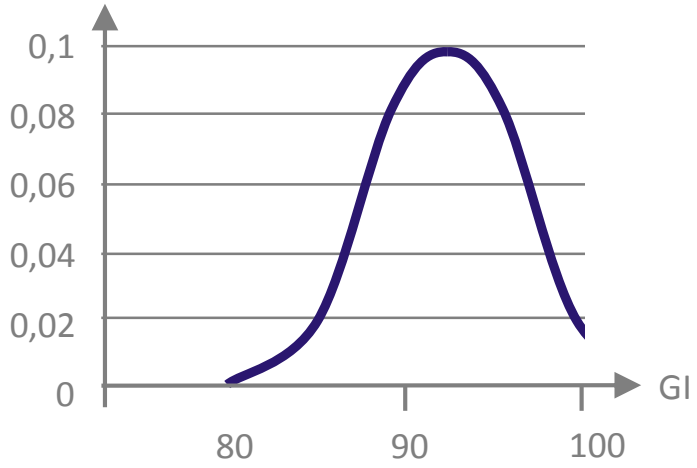
*Factor impacting efficacity

Therapeutic antibodies: Germinality Index (GI)

Germinality Index (GI) = Proportion of amino acids in V domain which are identical to human **germinal sequences**



GI distribution of 100 random human IgG



- Average GI: **~92%**
- 80% of human IgG exhibit a **GI > 88%**

(Thullier P, Chahboun S, Pelat T. MAbs. 2010 Sep-Oct;2(5):528-38)
(Pelat T et al. J Mol Biol. 2008 Dec 31;384(5):1400-7)

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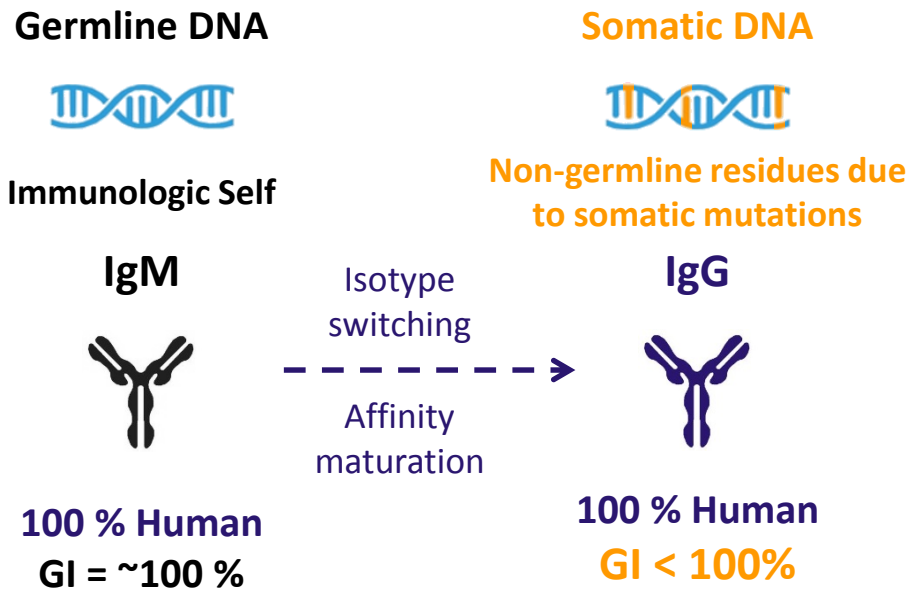

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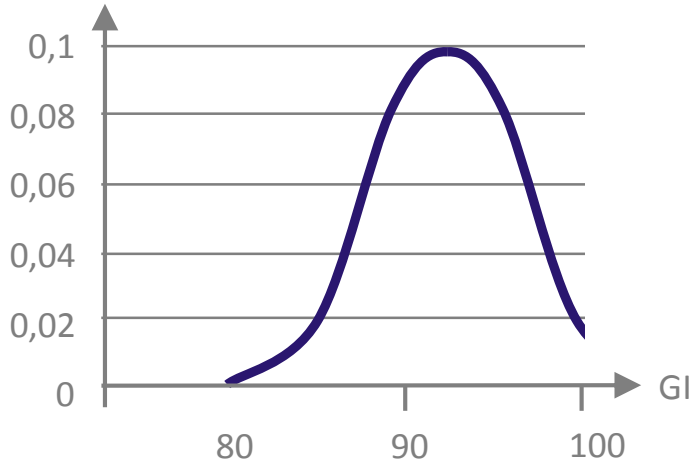

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Therapeutic antibodies (2nd and 3rd Generations): Side effects, ADA and GI

Antibody (INN)	Target	Strategy	ADA	GI
Briakinumab (ABT-874)	IL12/IL23	Human (Naïve Library)	Unknown	90
Ramucirumab, 1121B, IMC-1121B (CYRAMZA™)	VEGF R2		None Described	92
Ustekinumab (STELARA™)	IL12/IL23	Human (Tg mice)	YES	94
Canakinumab (ILARIS)	IL 1		None Described	83
Figitumumab, CP-751871	IGF1R		Unknown	96
Tocilizumab (Actemra)	IL 6R	Humanized	YES	88
Alemtuzumab, CAMPATH-1H, MABCAMPATH®	CD52		YES	82
Teplizumab, humanized OKT3	CD3		YES	79
Vedolizumab	A4B7 integrin		YES	87
Omalizumab, XOLAIR®	IgE		YES	85
Efalizumab, hu1124, RAPTIVA®	CD11a		YES	85
Bevacizumab, rhuMAb-VEGF, AVASTIN®	VEGF		None Described	85
Trastuzumab, HERCEPTIN®	HER2		YES	85
Pertuzumab, OMNITARG™, rhuMAB 2C4	ERBB2 (HER2)		None Described	84
Farletuzumab, M3, MORAb-003	FOLR1		Unknown	80

- Average GI for **humanized antibodies** is substantially low (~84%) with a high proportion of ADA
- Large GI amplitude for **fully human antibodies** (83%-96%)
- **Rational**: GI should be kept as high as possible to best mimic endogenous human IgG (**92%-100%**)

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