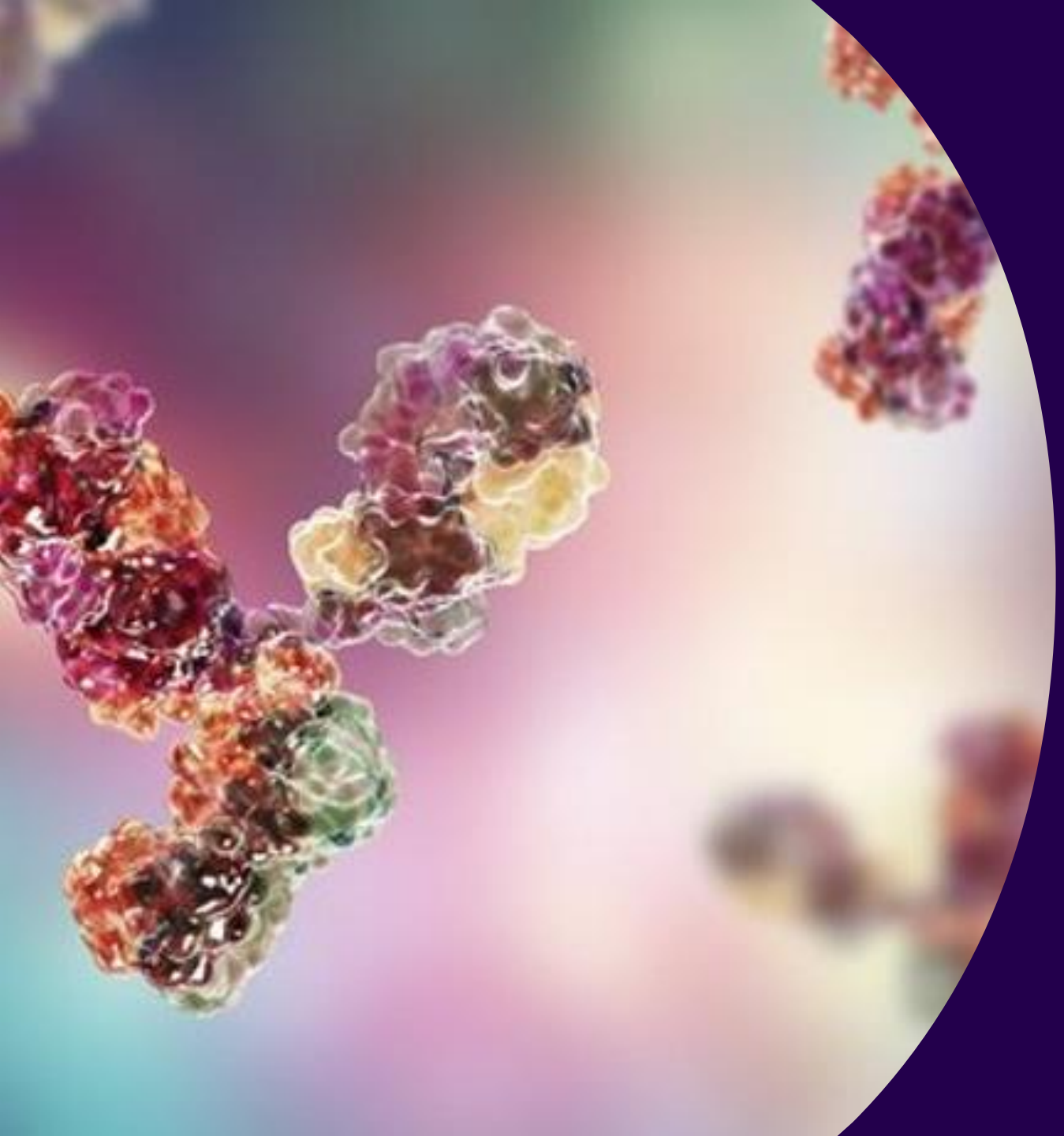

Strengths and weaknesses of non-animal derived therapeutic antibodies

Thomas Bouquin – Global Head Antibody Discovery / Large Molecule Research

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The Danish 3R Symposium 2022
November 8-9, 2022

Disclaimer

Thomas Bouquin – Global Head Antibody Discovery / Large Molecule Research, SANOFI

Expertise: +20 years in protein & therapeutic antibody discovery using both *in vivo* (wild type & transgenic mice, rats & chickens) and *in vitro* (phage display) platforms

Previous professional experience: Maxygen (Denmark & USA), Vipergen (Denmark), Symphogen, now Servier (Denmark)

Member of the European Animal Research Association (EARA) and the European Federation of Pharmaceutical Industries and Associations (EFPIA) response to antibody recommendation from ECVAM

Provides scientific expertise in therapeutic antibody matter to French Research Ministry office to help implementing the ECVAM directive

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Agenda

- Brief presentation of Sanofi's R&D organization and footprint
- Thesaurus & scope of the presentation
- Overview of the drug development process
- Therapeutic antibody discovery process
  - Antibody discovery platforms (*in vivo* & *in vitro*) – pros & cons
  - Animal usage across the antibody discovery process
  - Selection of the optimal modality and antibody discovery platform
- Initiatives to reduce animal usage at Sanofi
    - *Sanofi's Integrated Research and Testing Strategy (IRTS)*
    - *In vivo* antibody generation – transgenic mice and camelids
    - Preclinical safety
- Conclusion
- Acknowledgements

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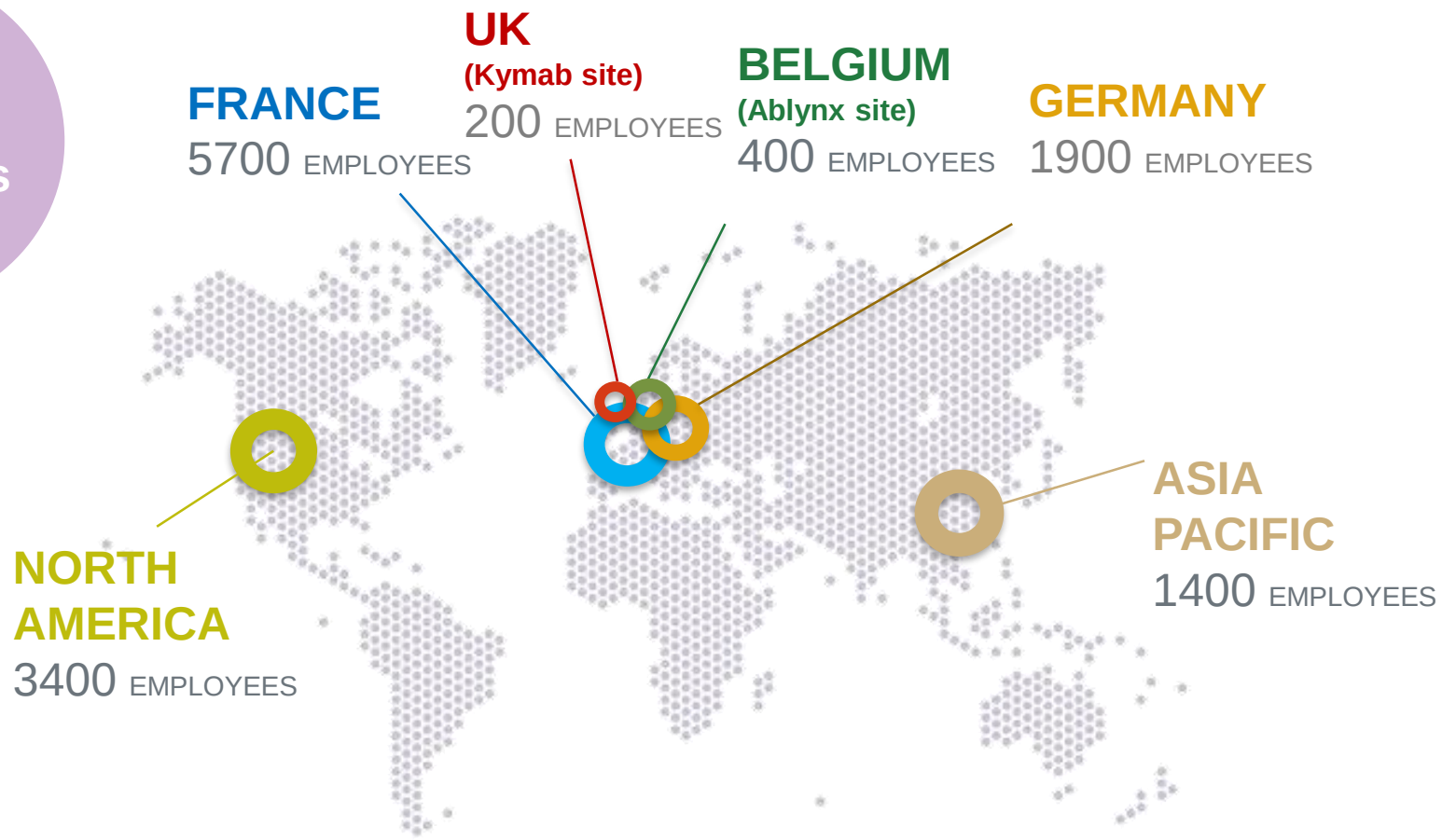
SANOFI R&D Footprint

15 000* employees committed to R&D

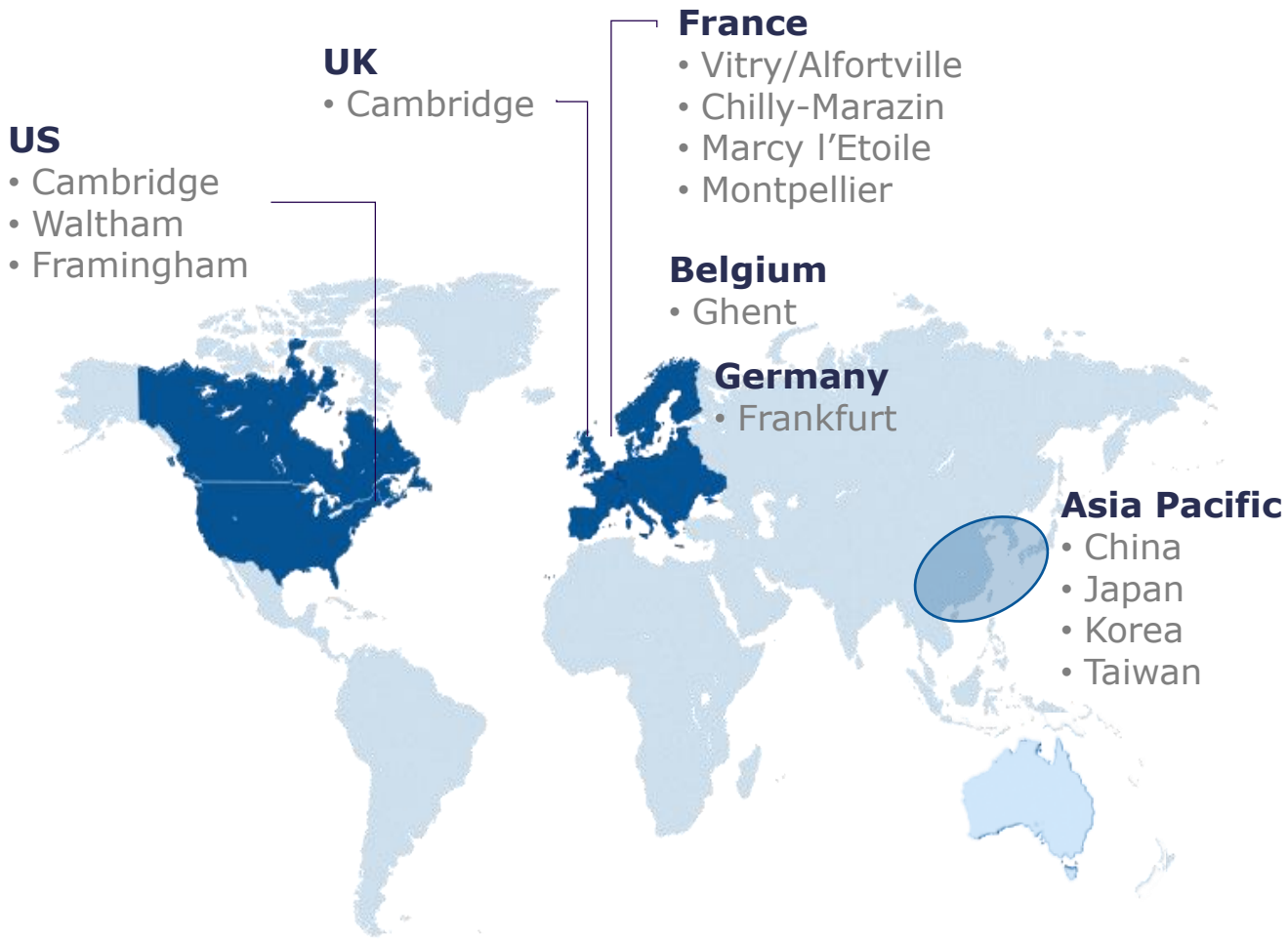
across **3** continents

in **4** hubs



** includes medical, IA and business unit functions working with R&D*

Our Research Organization



Therapeutic Areas

Immunology & Inflammation

Oncology

Rare & Neurologic Diseases

Vaccines

Research Platforms

Large Molecule Research (LMR)

Integrated drug Discovery

DMPK

Preclinical Safety

Translational In Vivo Models

Thesaurus and scope of this presentation

Antibody discovery
Antibodies can be used for numerous applications, including tool and therapeutic antibodies. This presentation will focus on therapeutic antibodies and derivatives during their research phase.

***In vitro* Ab technologies**: *in vitro* libraries are usually using phage display technology. They can originate from immunized animals (transgenic mice), naive immunoglobulin genes (from donors) or synthetic antibodies (in silico design). This presentation will focus on the libraries available at Sanofi.

***In vivo* Ab technologies**: Therapeutic antibodies can originate from various animal sources, including wild-type (rodents, camelids, birds, …) and transgenic (mice, rats, rabbits, chickens) animals. Therapeutic Abs can be either « polyclonal » or « monoclonals ». A majority of antibodies in the clinic are monoclonal. Polyclonal are usually related to specialty care when monoclonal Abs can not be considered. This presentation will focus on *therapeutic monoclonal and multispecific antibodies generated in transgenic animals*

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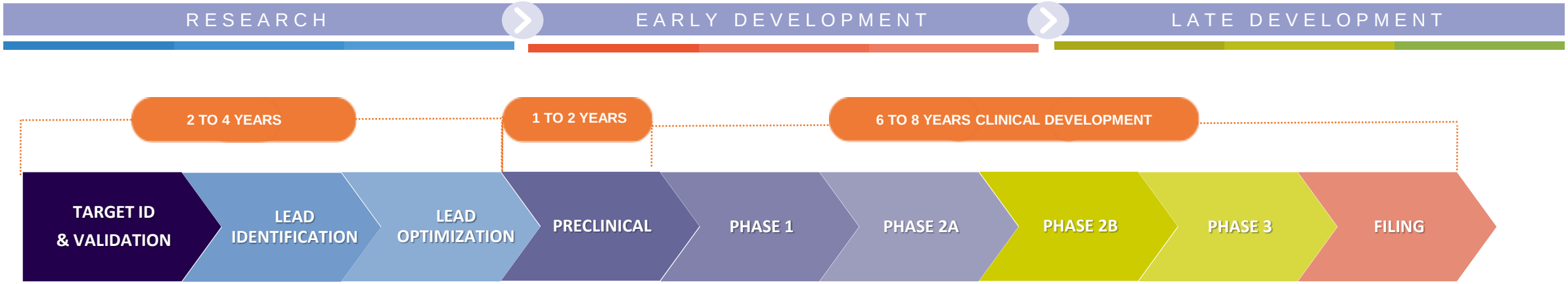
Overview of Drug Discovery and Clinical Development Process



Search for a drug target with evidence of relevance to disease
Which path ?

Search for molecule/ therapy acting on chosen target & with potential to be a drug
How to cure?

Validation of a drug candidate
Potential & efficient cure?

Check of safety, tolerance, efficacy & various stages of development on cells & animals models

Studies with samples of healthy volunteers to check safety tolerability

Studies with patients to check the drug candidate is efficient vs initial hypothesis

Studies with sick patients to check the drug candidate cures or prevents a disease

Studies with larger groups to assess safety, efficacy & effectiveness vs placebo or other treatments

Application to the regulatory authorities of a country requesting approval of a drug candidate

Focus of the presentation

Internal efforts to reduce animal usage will be presented

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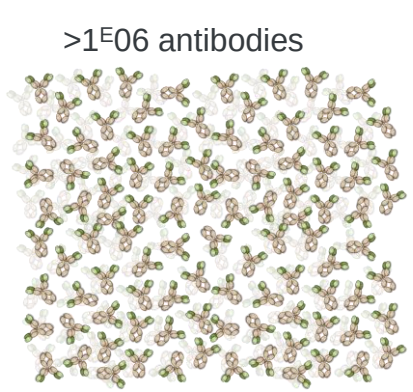
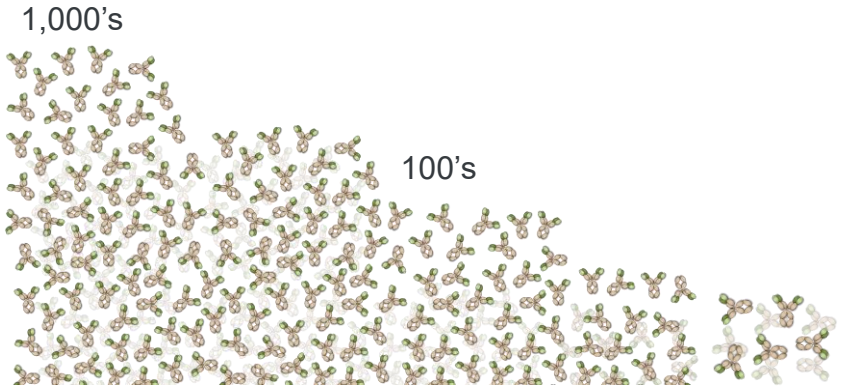
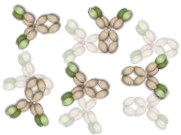
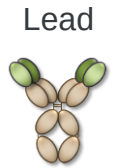
Therapeutic Antibody Discovery Process

Overview of Sanofi's antibody platforms and discovery process up to lead identification









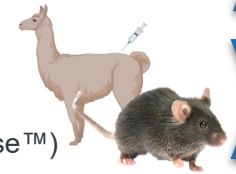
Antibody platforms

Antibody generation | **Antibody screening** | **Lead identification** | **Clinical development**

Phage display (commercial & proprietary libraries)

Phage display selection → Sequencing & Selection → HTP antibody expression → Antibody screening → Milligram-scale production → *In vitro* functional validation → *Antibody engineering (optional)* → Gram-scale production / Developability

Camelids & transgenic mice (Trianni™, Kymouse™)



immunization → Antigen-specific antibody sequence capture from B cells → In vivo functional evaluation

PK / PD

Tox

In silico-driven antibody discovery

In silico-driven antibody generation (future)

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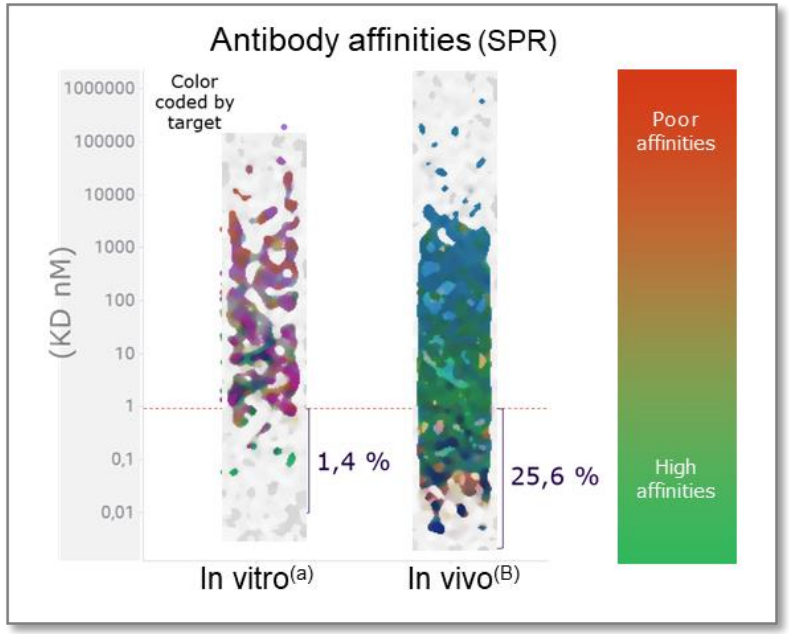
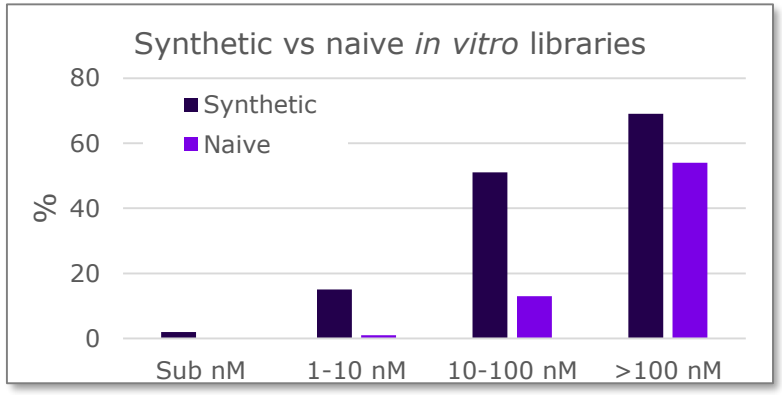
in vitro vs *in vivo* antibody platforms

Antibody affinities





		#clones evaluated	% < 1 nM high affinities	% < 10 nM medium affinities
In vitro (Phage display)		501	**1.4**	18.2
In vivo	Trianni™	3231	**25.6**	48.5
	Other (undisclosed) transgenic mouse used by competitors and evaluated H2H with Trianni™	270	**14.8**	25.2

- The probability to identify high affinity antibodies is ~20 fold lower when using *in vitro* as compared to *in vivo* (Trianni™) platform
- High affinity antibodies were identified for all targets using the *in vivo* platform, but not from *in vitro* libraries
- Naïve *in vitro* libraries exhibit a lower percentage of medium and high affinity Abs than synthetic library
- When high affinity is an important feature of the **therapeutic** antibody, *in vitro*-derived Abs will likely require an extra affinity maturation step

(a) Commercial and Sanofi's synthetic *in vitro* libraries
(b) Trianni™

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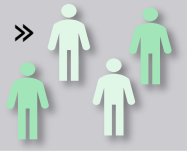
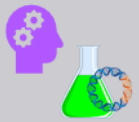
SANOFI's libraries for *in vitro* human antibody discovery

	Immune	Naive	SaVANT	Specifica
Origin	Sanofi	Commercial (undislosed)	Sanofi	Specifica
Antibody Format	Fab	scFv	Fab	Fab & scFv libraries
Antibody source	Transgenic mice (Trianni™) 	Human « naive » donors 	Synthetic 	Synthetic 
Light chain	kappa	Kappa & lambda	Kappa & lambda	Kappa & lambda
Library size	10^8	10^9	10^{10}	10^{10}
Pros	• High affinity antibodies (similar to *in vivo* platform process)	• Used in parallel with other *in vitro* approaches as backup	• High success rate to identify binders • Favorable developability profile (by design)	Expected: • High frequency of high affinity antibodies • Favorable developability profile (by design)
Cons	• « Scrambled » heavy and light chain variable regions • Longer process than *in vivo* platform process	• Poor success rate to identify binders • Low affinities (affinity maturation is required)	• Lower frequency to identify high affinity antibodies than *in vivo*-derived antibodies	None anticipated

Sanofi/Specifica. Press release

Pros & cons of *in vitro* and *in vivo* antibody platforms

SANOFI's platforms

	Wild-type mice	Transgenic mice	In vitro	Comment
Adapted to conserved targets?				Highly conserved target are poorly immunogenic
Time to reach primary hits (i.e binders)				*In vitro* libraries are ready to screen, whereas *in vivo* approaches require immunization
Time from primary hits to lead				• Lengthy humanization required for wild-type animal-derived Abs. • Affinity maturation (more often) required for *in vitro*-derived hits.
Antibody affinities				• Higher probability to find high affinity Abs from *in vivo*-derived Abs • Affinities of *in vitro*-derived Abs are linked to the library design and screening process
Developability				• Wild-type animal-derived Abs require humanization before being developed as therapeutic. Most big Pharma have access to transgenic platforms • Developability of *in vitro*-derived Abs is related to library design. Sanofi's proprietary libraries are designed to exhibit good developability.
Platform access				Licence or internal platform generation cost for transgenic animals or **high-quality** *in vitro* libraries is high

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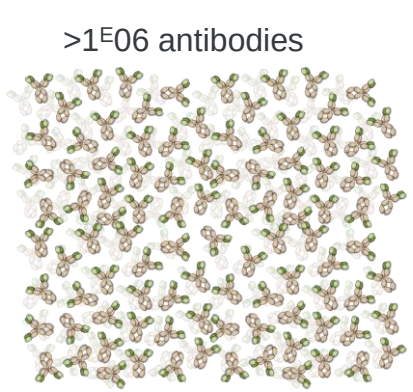
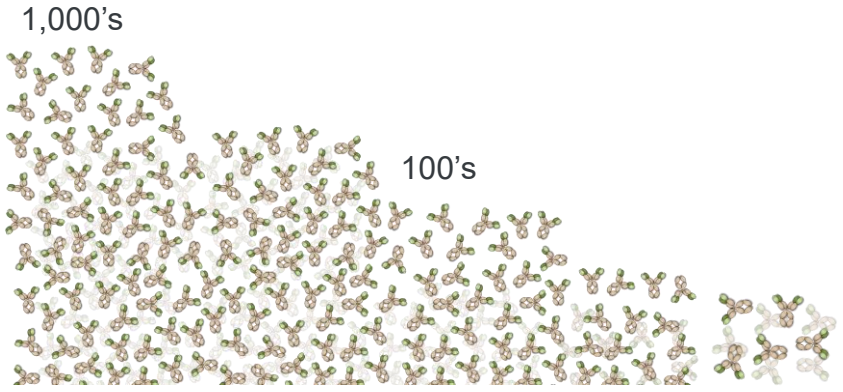
Therapeutic Antibody Discovery Process

High throughput and milligram-scale production for supporting hit identification





Antibody platforms

	Antibody generation		Antibody screening					Lead identification	Clinical development
Phage display (commercial & proprietary libraries)	Phage display selection	Sequencing & Selection	HTP antibody expression	Antibody screening	Milligram-scale production	*In vitro* functional validation	*Antibody engineering (optional)*	Gram-scale production / Developability	
Camelids & transgenic mice (Trianni™, Kymouse™)	immunization	Antigen-specific antibody sequence capture from B cells						In vivo functional evaluation	
In silico-driven antibody discovery	In silico-driven antibody generation (future)							PK / PD	
								Tox	

High throughput (microgram-scale) and milligram-scale antibody production

- Performed on robotic platforms using recombinant technologies (expression in mammalian cells in culture medium)
- No animals are used at this step. No ascites!

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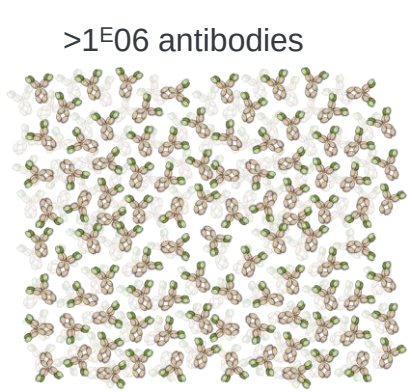
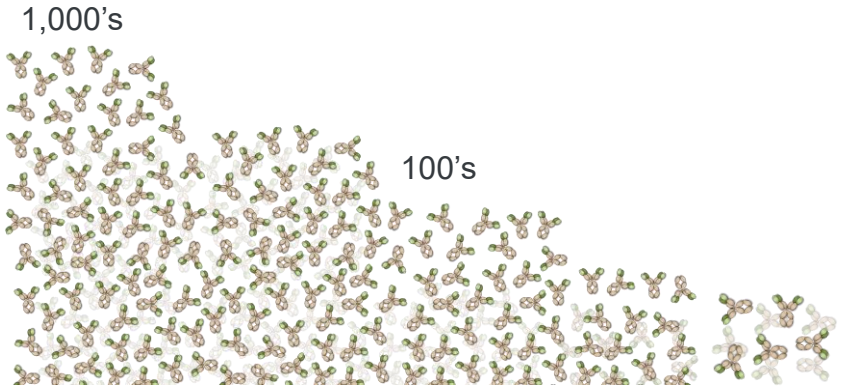
Therapeutic Antibody Discovery Process

High throughput antibody screening





Antibody platforms

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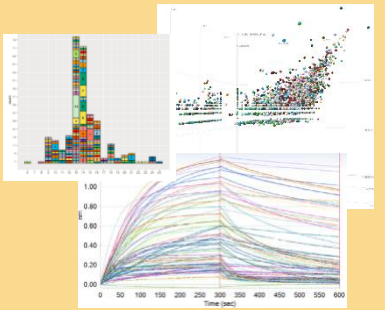
								Tox	

Antibody screening

- Antigen binding confirmation
- Cross-reactivity with relevant species, e.g Cynomolgous monkey
- Binding strength (affinity)
- Ligand/receptor blocking
- Function (e.g. reporter cells)
- Epitope binning / domain mapping

- No animals are used at this step.



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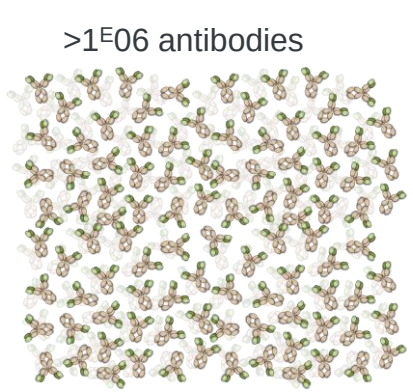
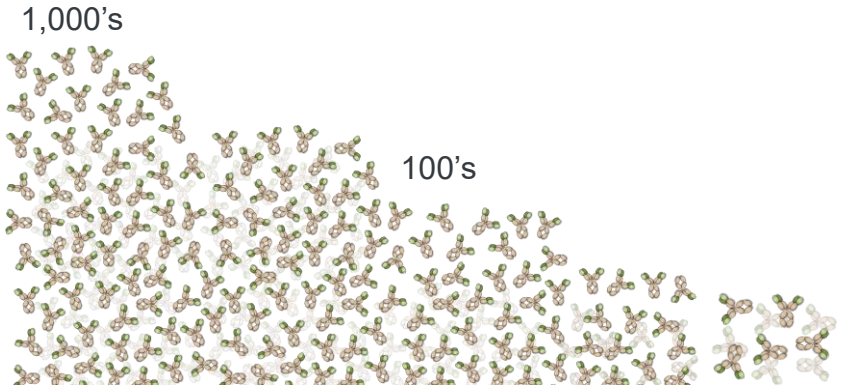
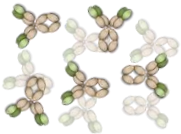
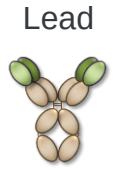
Therapeutic Antibody Discovery Process

High throughput antibody screening









Antibody platforms

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Camelids & transgenic mice (Trianni™, Kymouse™)	immunization	Antigen-specific antibody sequence capture from B cells						In vivo functional evaluation	
In silico-driven antibody discovery	In silico-driven antibody generation (future)							PK / PD	
								Tox	

Antibody engineering (optional)

- Can be necessary to increase the efficacy or potency of the antibody (e.g. affinity maturation) or remove CMC-related liabilities that could impede preclinical development: production levels, stability during process development or upon storage, etc
- Lengthy, labor intensive and unpredictable process that can be performed only on a small number of hits → should be avoided when possible

- No animals are used at this step.

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Therapeutic Antibody Discovery Process

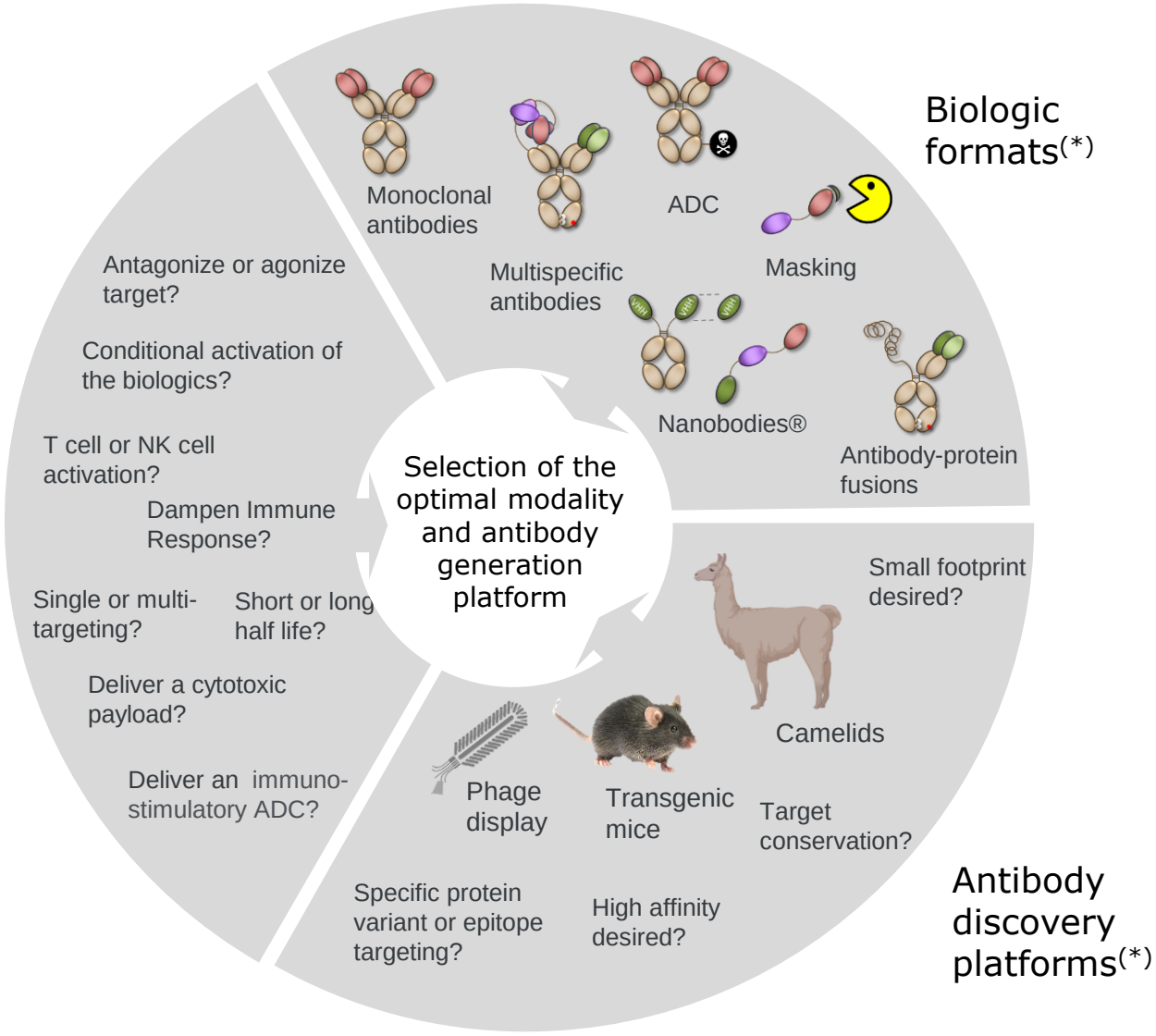
Lead identification

Lead identification

Gram-scale production / Developability	In vivo functional evaluation	PK / PD	Preclinical safety
Gram-scale production • Mammalian cell-based production in culture medium • No animals are used at this step.	**In vivo functional evaluation** • Typically using (humanized) mice models for the disease	**PK** • Performed in Tg32 mice - FcRn-/- hFcRn (32)	**Preclinical safety** • Performed in relevant animal models
			

Therapeutic Antibody Discovery Process

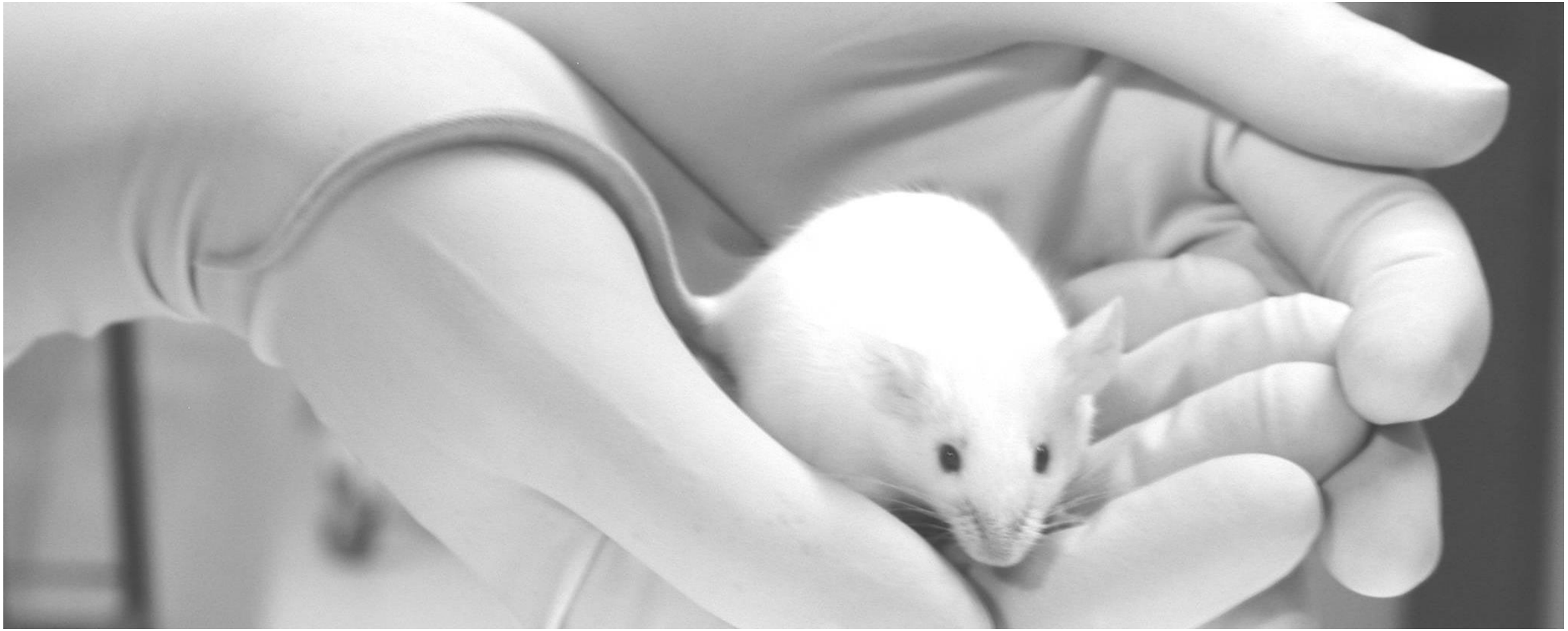
Antibody platforms and biologics format combinations to fight disease



- Sanofi's formats for biologics include monoclonal, multispecific antibodies and Nanobodies®, Fc effector enhanced or attenuated, ADC, antibody fusion proteins, PK modulation and masking technologies
- The choice of the biologic modality is driven by (1) the desired mode of action / target product profile and (2) fitness of the antibody discovery platforms for given parameters

(*) Not exhaustive list

Initiatives to reduce animal usage at Sanofi



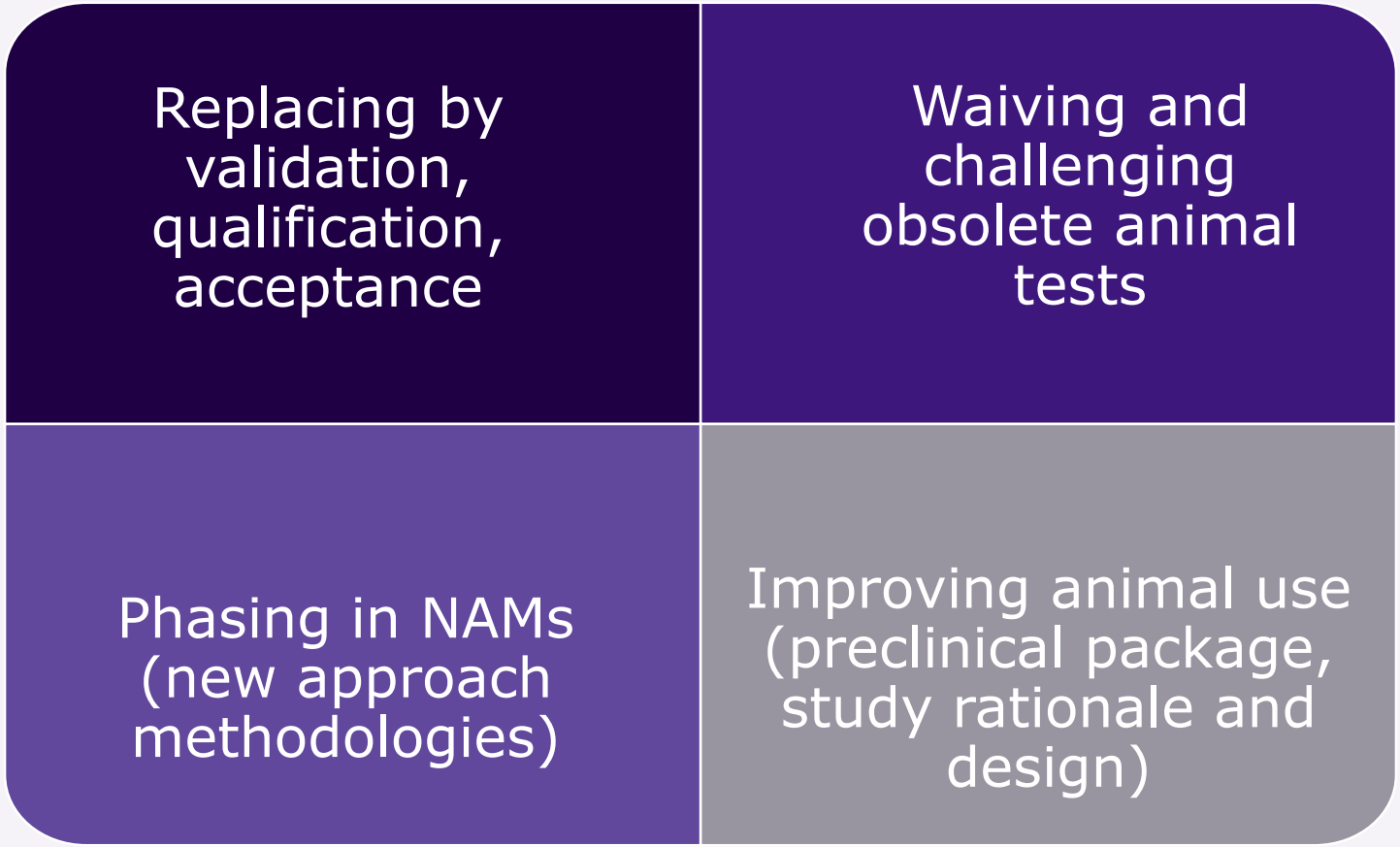
What is the Integrated Research and Testing Strategy (IRTS)?

IRTS is Sanofi strategy that lays out our guidelines to affirm rigorous, state-of-the-art science as key criteria to select the best available, feasible, and translatable models to address scientific questions and adhere to regulatory requirements, most importantly with the primary aim to relieving Sanofi of toward reliance on live animals.

How will we do it?

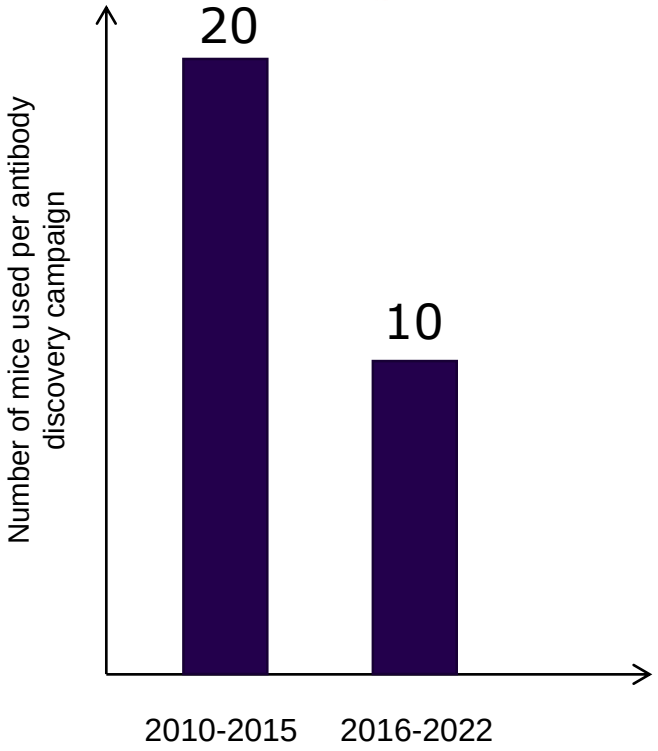
Objective: very significant global reduction over a 10 years period

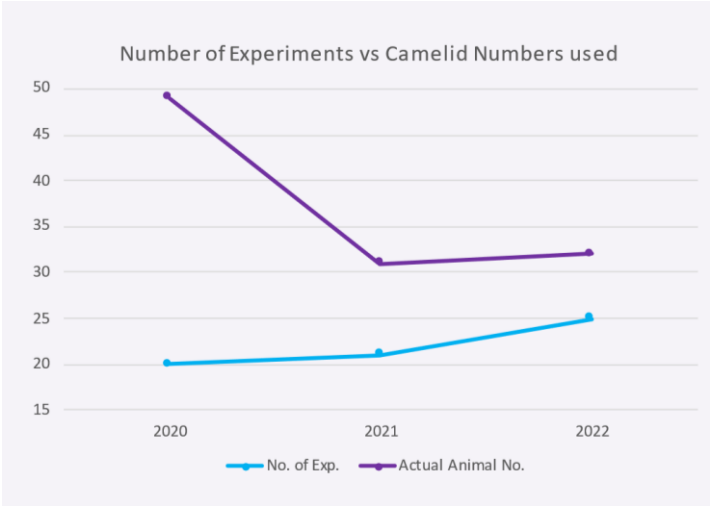
- Up to 50% reduction
- Between 2020 and 2030
- Internal and external use



Antibody Discovery

Determination of the optimal number of immunized animals





Reduction in number of immunized animals/experiment was made possible by:

- Improving immunization procedure to obtain 100% animals exhibiting good serum titers
- Determining the antibody diversity obtained per animal, then using only the number of animals necessary to capture the target diversity
- Performing more systematically *in vitro* discovery in parallel to *in vivo*
- Improving the overall antibody discovery process by capturing more antibodies from each animal
- Poly-antigen immunizations

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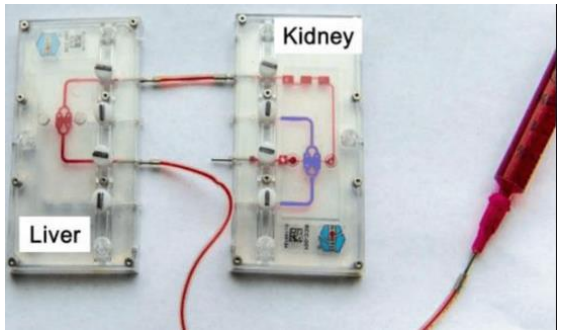
https://www.rockstepsolutions.com/blog/the-3rs-of-humane-animal-research/

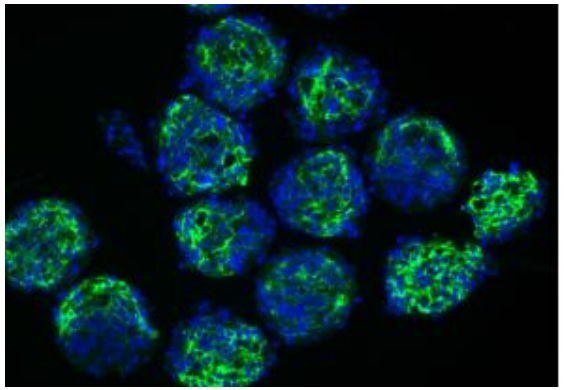
PK and preclinical Safety

Advanced In Vitro Human Models

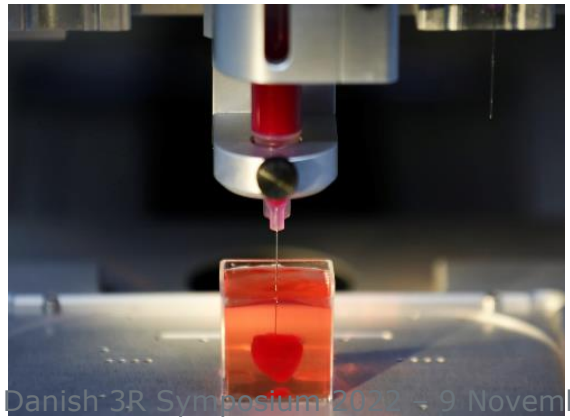
- **Organ-on-a-chip**: Microfluidic devices (2D/3D) with physiologically relevant perfusion



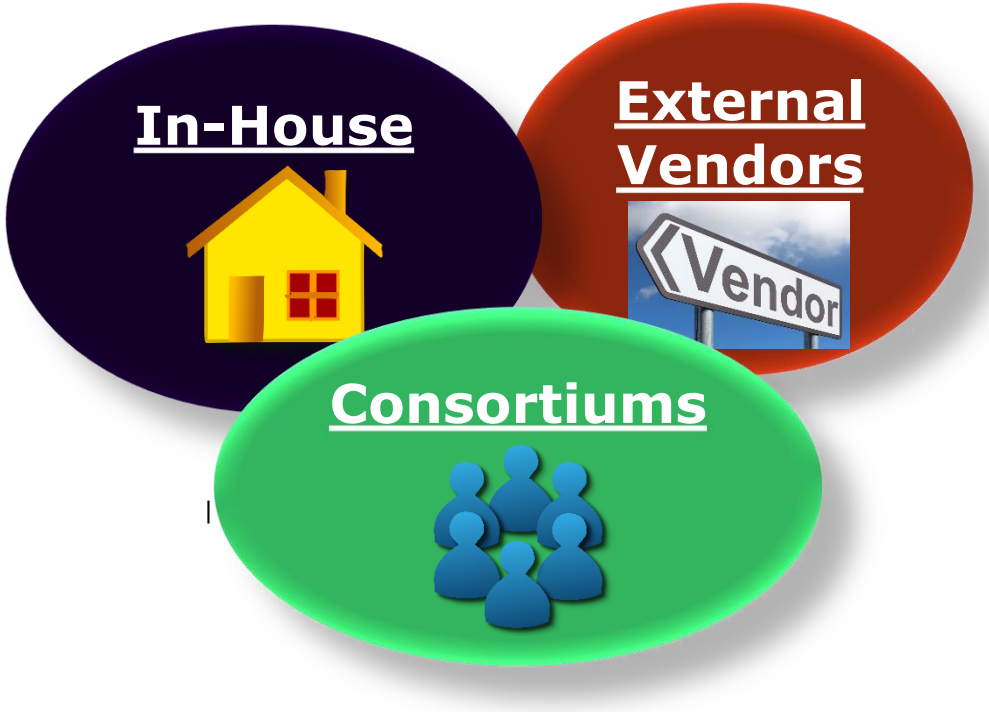
- **Organoid/spheroid**: 3D single or multi-cell aggregates of stem or primary cells from a single organ



- **3D Printed**: Using 3D bio-orienting techniques to combine cells in a 3D matrix that mimics in vivo tissue



Combined Efforts to Ensure Faster Development and Adoption



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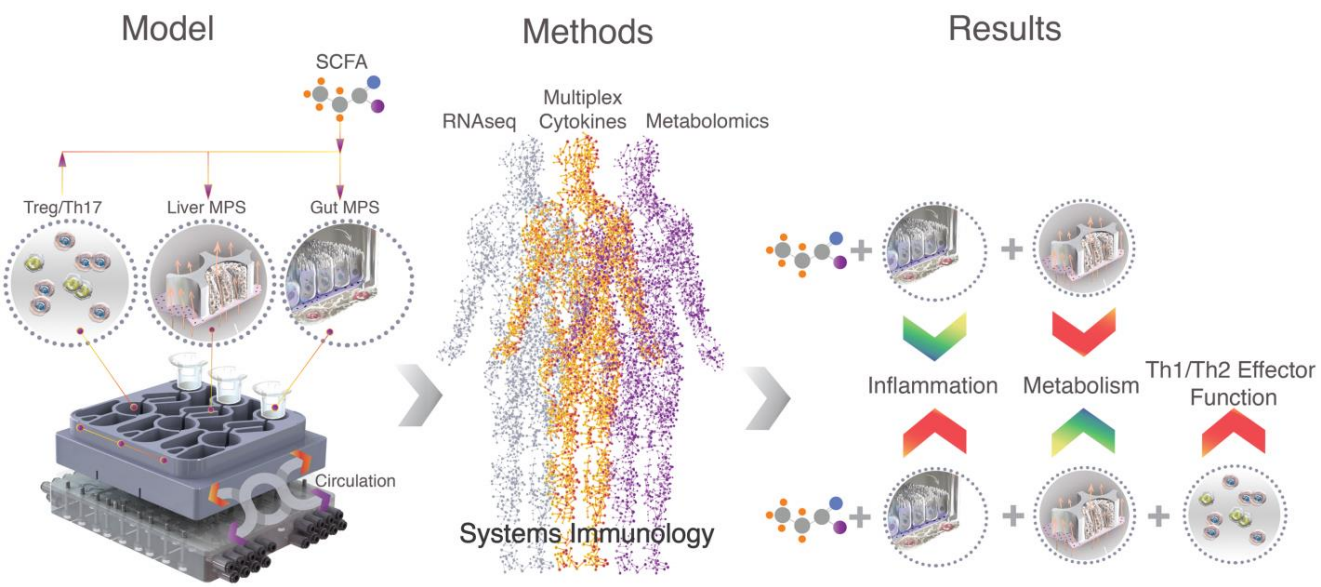
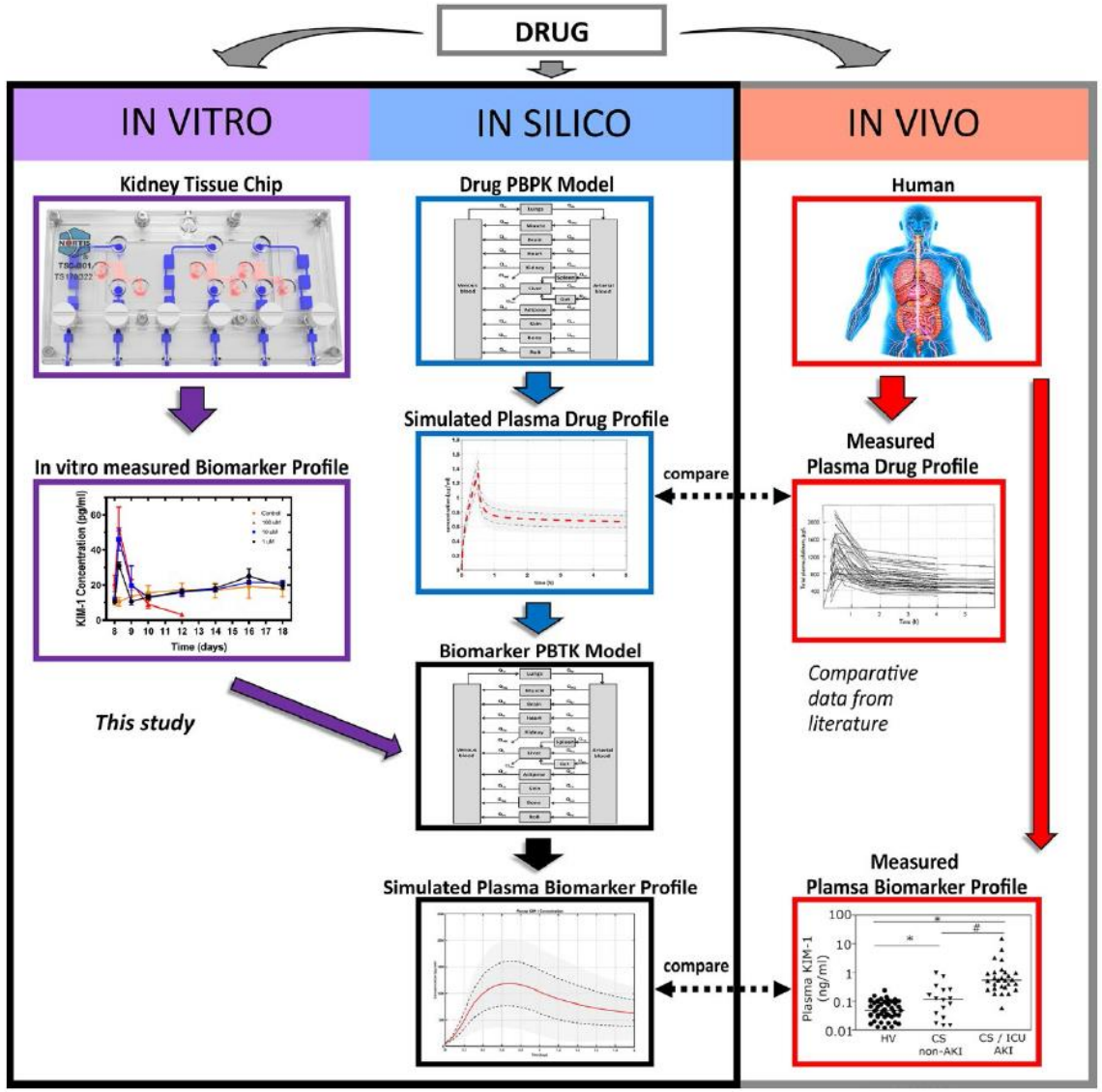
PK and preclinical Safety

Future Models of Drug Development



Trapecar et al., BioRxiv. 2019

In vitro modeling of complex multi-organ cross talk using human material, combined with advanced in silico modeling, will reduce animal use and enable better prediction of human response.



Maass et al., Pharmacometrics Syst. Pharmacol., 2019

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Conclusion

- Sanofi uses state of the art *in vitro* and *in vivo* technologies for generating therapeutic antibodies
- *In vitro* and *in vivo* platforms are complementary: *In vitro* technologies are better suited for conserved targets while transgenic mice provide a larger number of primary hits and a much higher probability to identify high affinity antibodies
- Next-generation *in vitro* libraries have the potential to replace transgenic animals if/when they will address the limitations of the current libraries
- Animal usage for antibody generation is very limited (10 mice or 2 camelids / target) and was optimized to obtain an optimal diversity from a small number of immunized animals
- Sanofi is committed to very significantly reduce the number of animals used in non-clinical and clinical research through the Integrated Research & Testing Strategy (IRTS), a novel paradigm going beyond the 3Rs principles

Aknowledgments

Large Molecule Research
Klervi Desrumeaux
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Nicolas Maestrali
Nikolai Suslov
Tarik Dabdoubi

Pre-clinical Safety
Karissa Adkins

Global Compliance and Policy,
Translational in vivo Models (TIM)
Elodie Bouchoux
Nicolas Dudoignon

Chief Veterinary Officer
Thierry Decelle