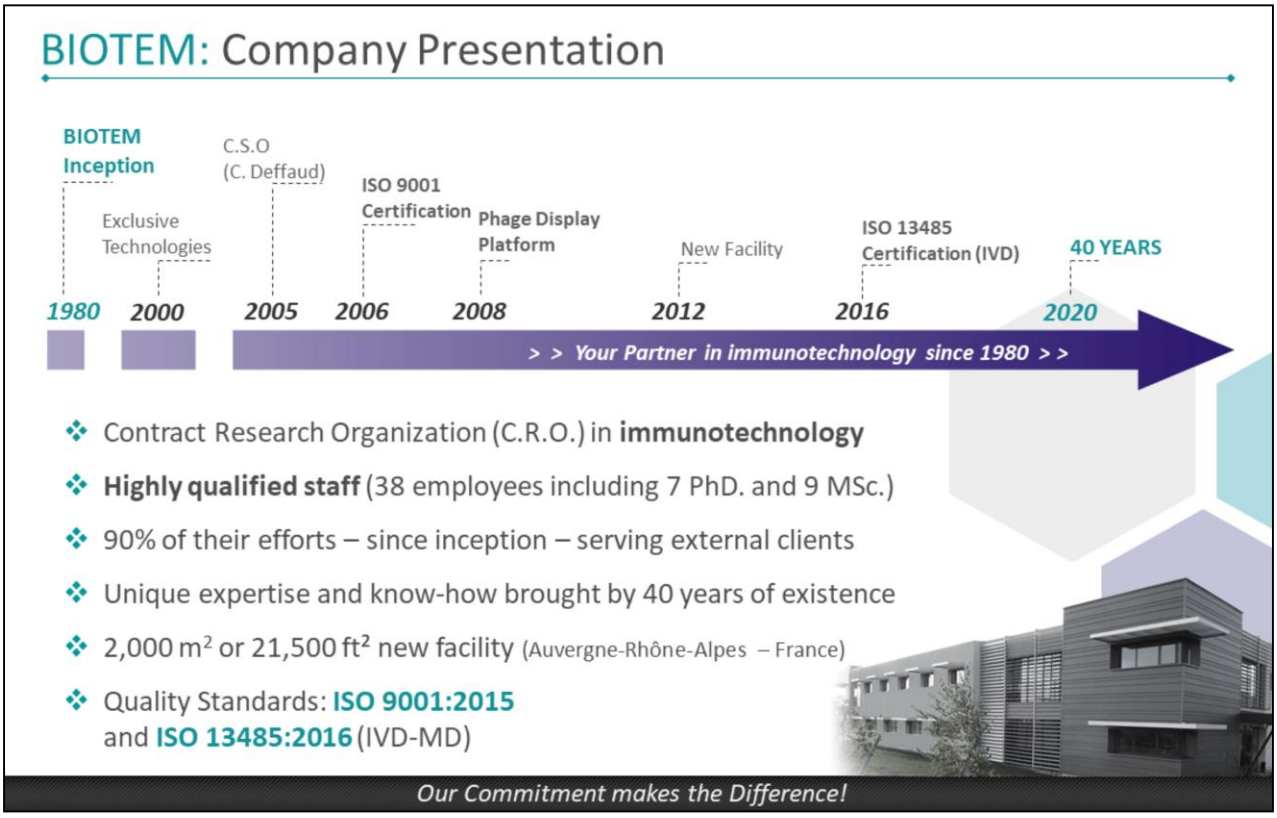
BIOTEM proposes the exclusive Ultimate Humanization®Platform for the discovery of new lead candidates.

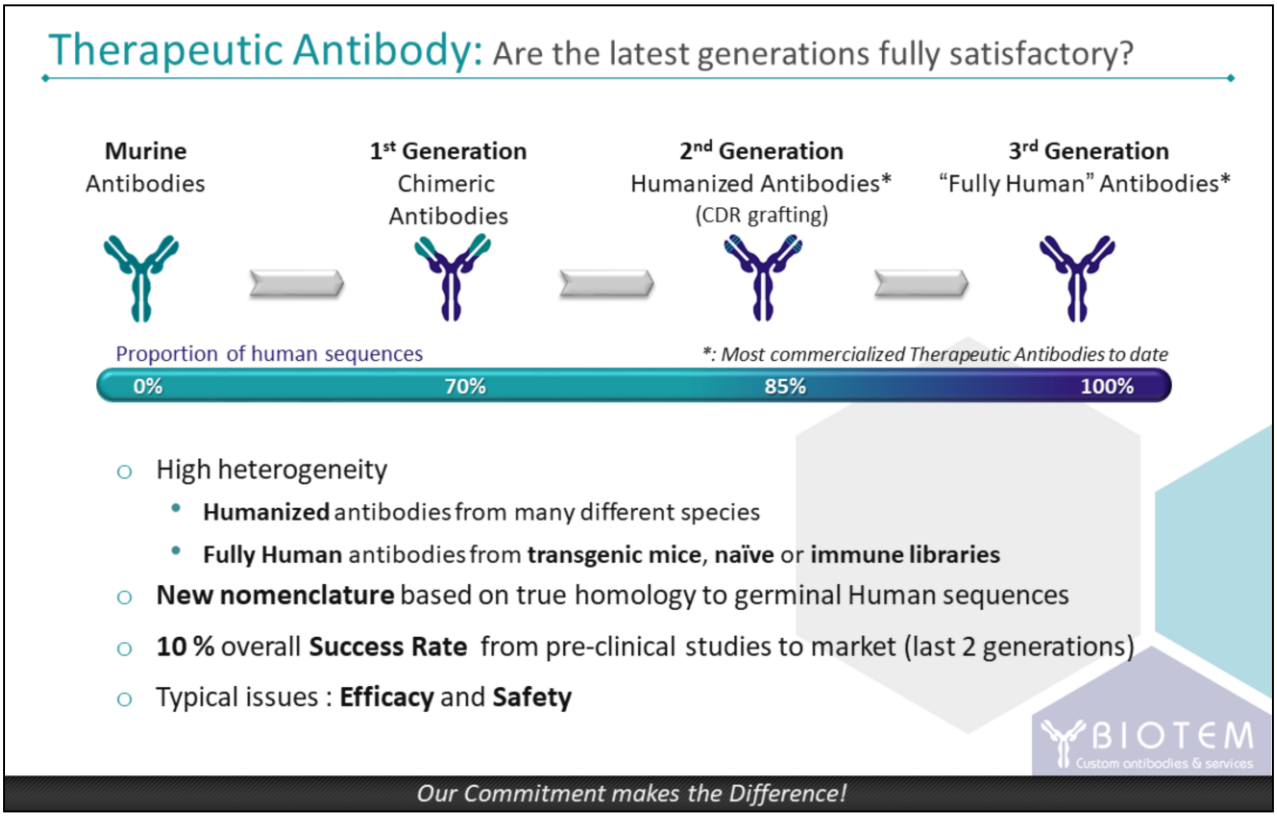
This platform is an integrated solution for the development of biobetter antibodies with high intrinsic qualities.



BIOTEM is a Contract Research Organization specialized in immunotechnologies for 40 years.

More precisely the company develops and produces antibodies & immunoassays for R&D and diagnostic applications.

BIOTEM also proposes a large panel of strategies for the development of therapeutic monoclonal antibodies at early discovery phases.



One of the main questions our clients usually ask us is "how can we efficiently develop biobetter antibodies?" … "Are the latest generations fully satisfactory?"

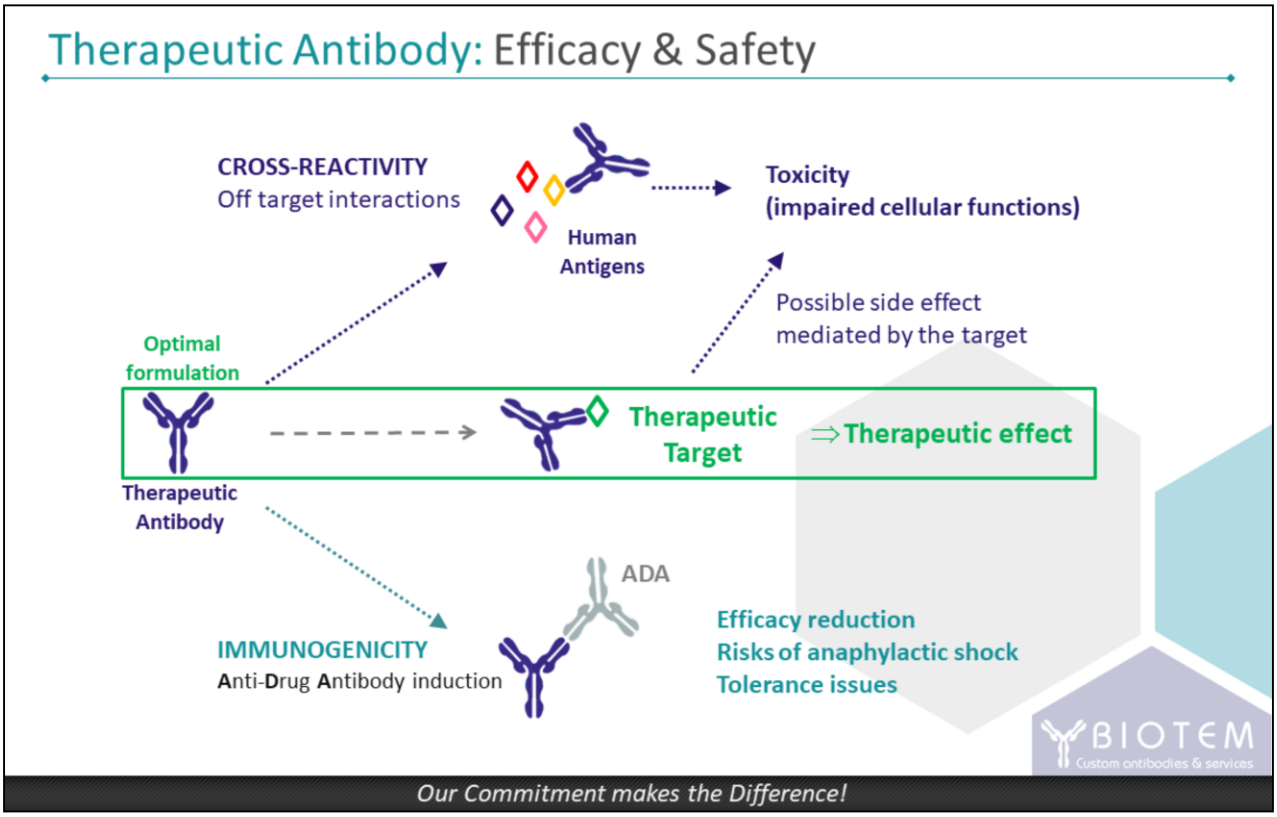
Just to remind you briefly. There have been 3 generations of therapeutic antibodies all based on different technologies: the chimeric, the humanized and the fully human antibodies, all aiming at increasing the proportion of human sequences in order to reduce immunogenicity issues.

At the same time this "human sequence" concept has not been clearly defined. How can we scientifically quantify the humanness degree of one antibody?

This is not an easy question!

Anyway, at BIOTEM we have our own idea on this and we use the Germinality Index (GI) to quantify the humanness degree. We will come back on this concept later on.

In any case with the last 2 generations there is only 10% success rate from pre-clinical phases to the market. Typically many antibodies fail for efficacy and/or safety reasons.

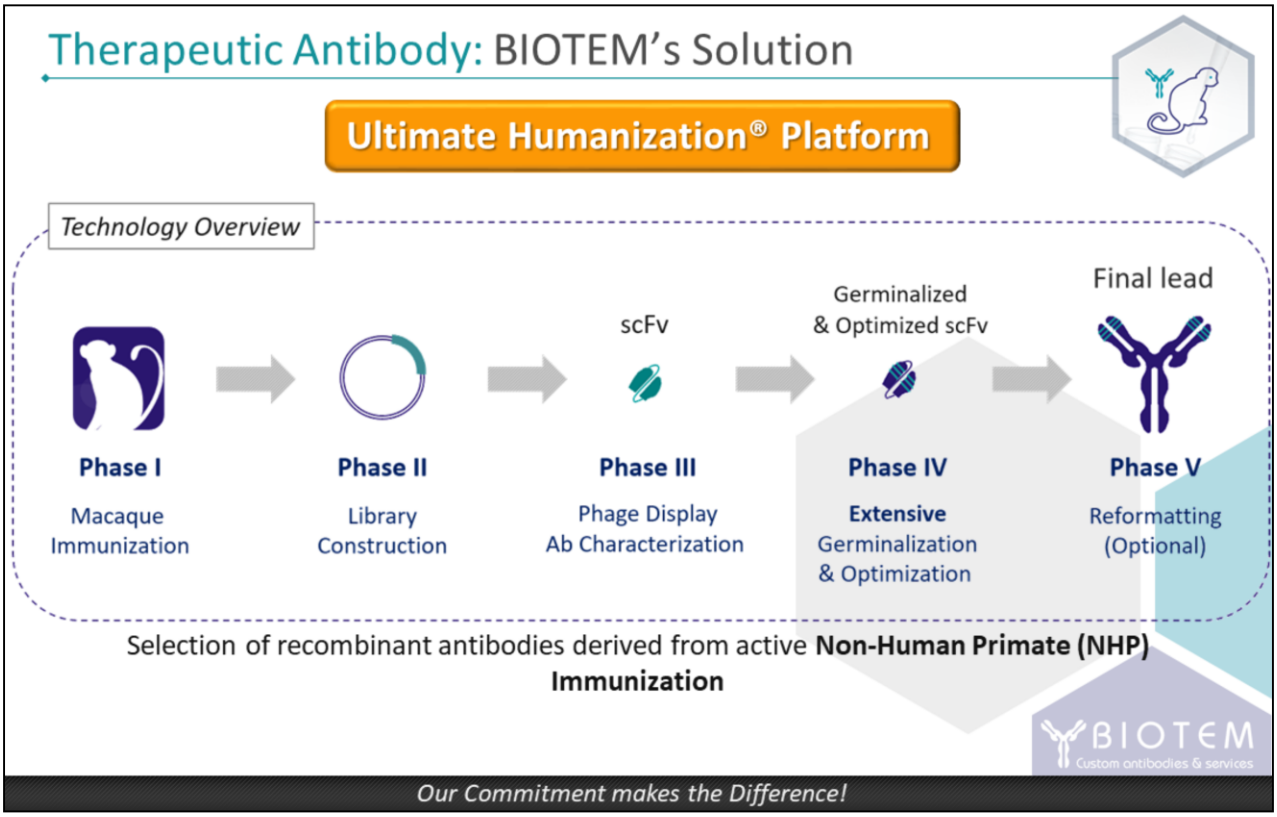


In a perfect world therapeutic antibodies would only bind to their therapeutic targets leading to the therapeutic effect.

However as for any biological interaction, the specificity is never perfect and therapeutic antibodies might also bind to other molecules… this is called “off target interaction” and this can impair important cellular functions leading to toxicity.

Therapeutic antibodies might also induce specific immune responses with the production of Anti Drug Antibodies (ADAs). These ADAs might reduce efficacy as well as compromise safety…

So, coming back to the first and crucial question: how can we develop better antibodies, our answer is to generate molecules using a technology that could lower both the risk of toxicity as well as the risk to generate ADAs.



Here you have a brief overview of the technology.

Our solution is to derive therapeutic antibodies from Macaque using active immunization, molecular library construction, phage display and a really **exclusive process** that we call **Extensive Germinalization** and we will explain this process in detail later on and even show a case study.

We also include state-of-the-art antibody optimization as well as a last reformatting phase in order to best suit with the intended application and mechanism of action.

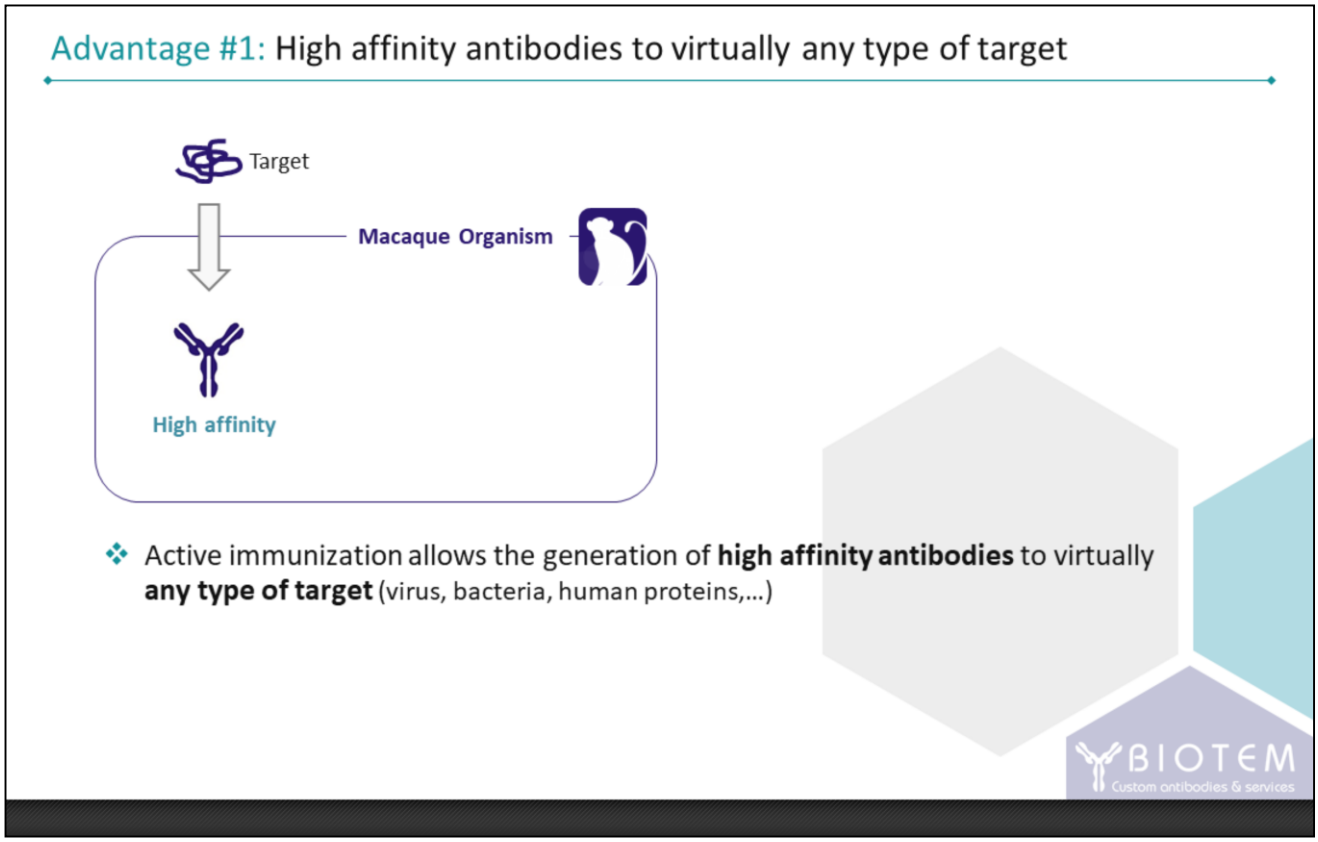
We start by an active immunization of 1 or 2 macaques per program. From the immunized animals we then generate "immune" molecular libraries of antibodies. Such libraries are highly focused on the immunizing target (as opposed to synthetic and naïve libraries).

Using Phage Display technology we isolate unique antibody binders to the therapeutic targets (those binders are in an scFv format meaning that only the variable and binding regions of the antibody is produced).

Then the binders are optimized and modified in order to make them resemble to real human antibodies in a process called germinalization, an extensive humanization strategy. This process is extremely efficient because macaques and humans are genetically extremely closed. We will latter describe it in more details.

Lastly the optimized binders might be reformatted in order to better suits the intended application.

Basically this overall strategy has 3 main advantages

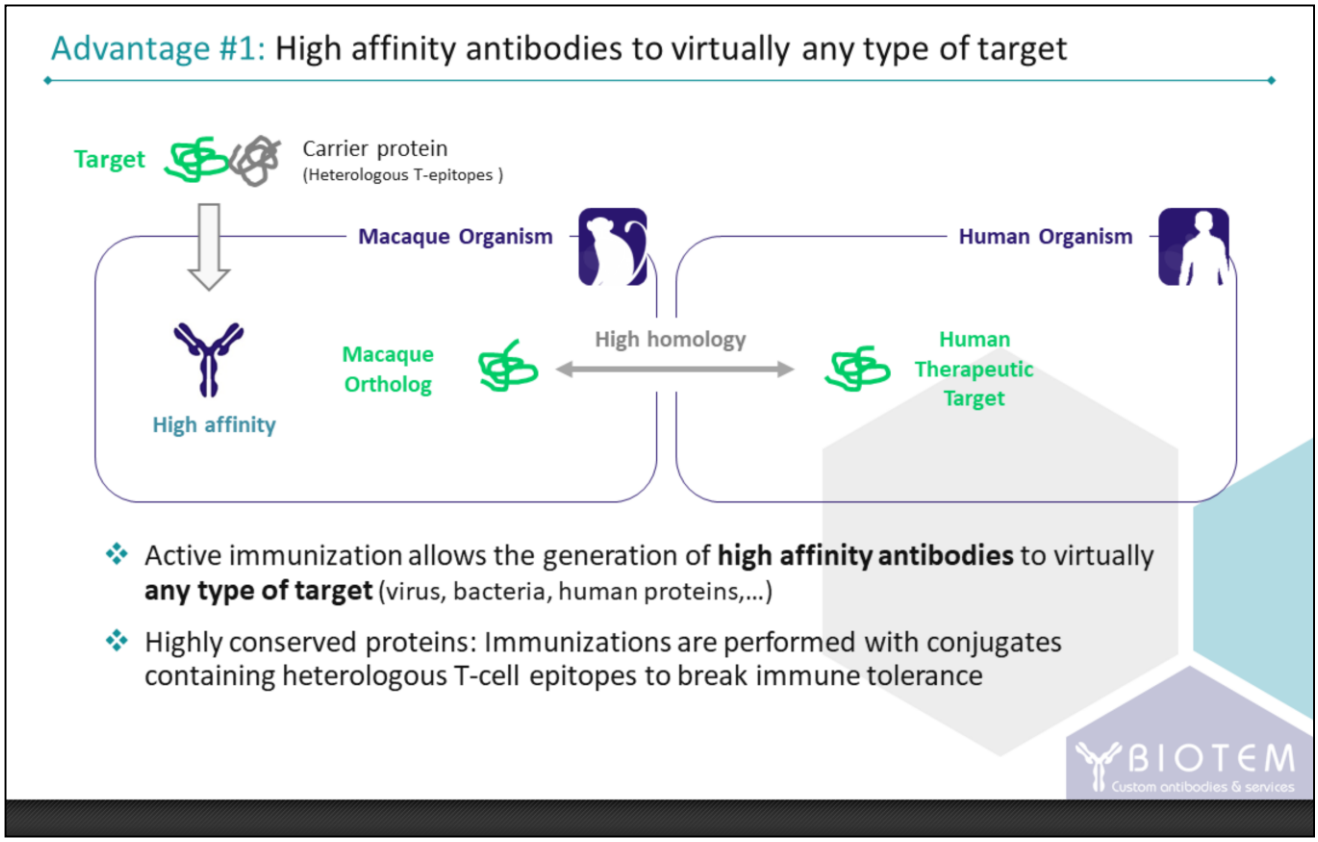


The 1st advantage is quite basic but very useful.

Active immunization allows to generate high to very high affinity antibodies (with Kd in the picomolar range).

Active immunization is also very straightforward and you can easily develop antibodies to virtually any type of target (virus, bacteria, human proteins,…)

But is it also efficient for human proteins which are very conserved as well as low immunogenic targets?

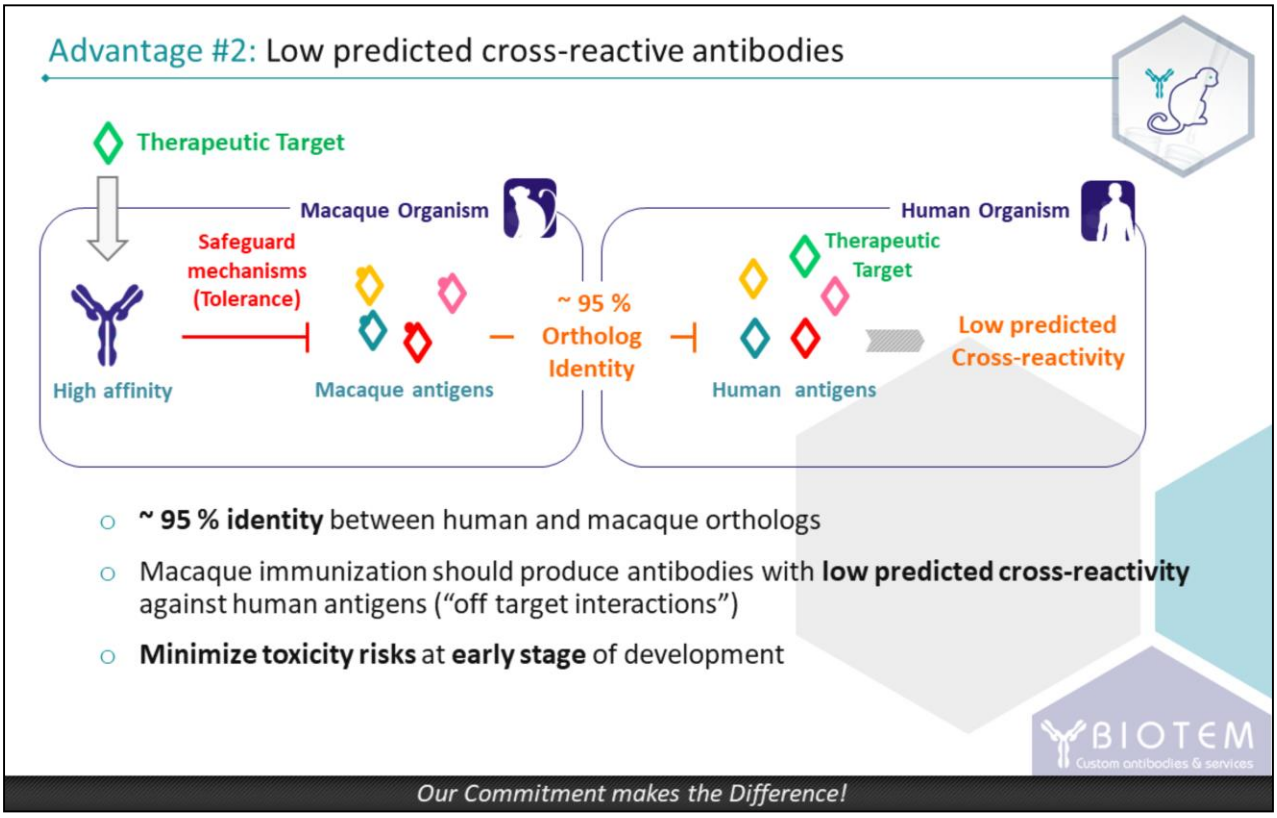


It fact it is quite easy to break tolerance and to increase immunogenicity to a specific target.

Here you have the example of a conserved target in green.

In order to increase immunogenicity the target is first conjugated to a carrier protein (in grey) in order to bring heterologous T-cell epitopes.

This strategy is very effective and we very commonly generate high affinity antibodies with a large epitope coverage to targets that are even 100 % conserved.

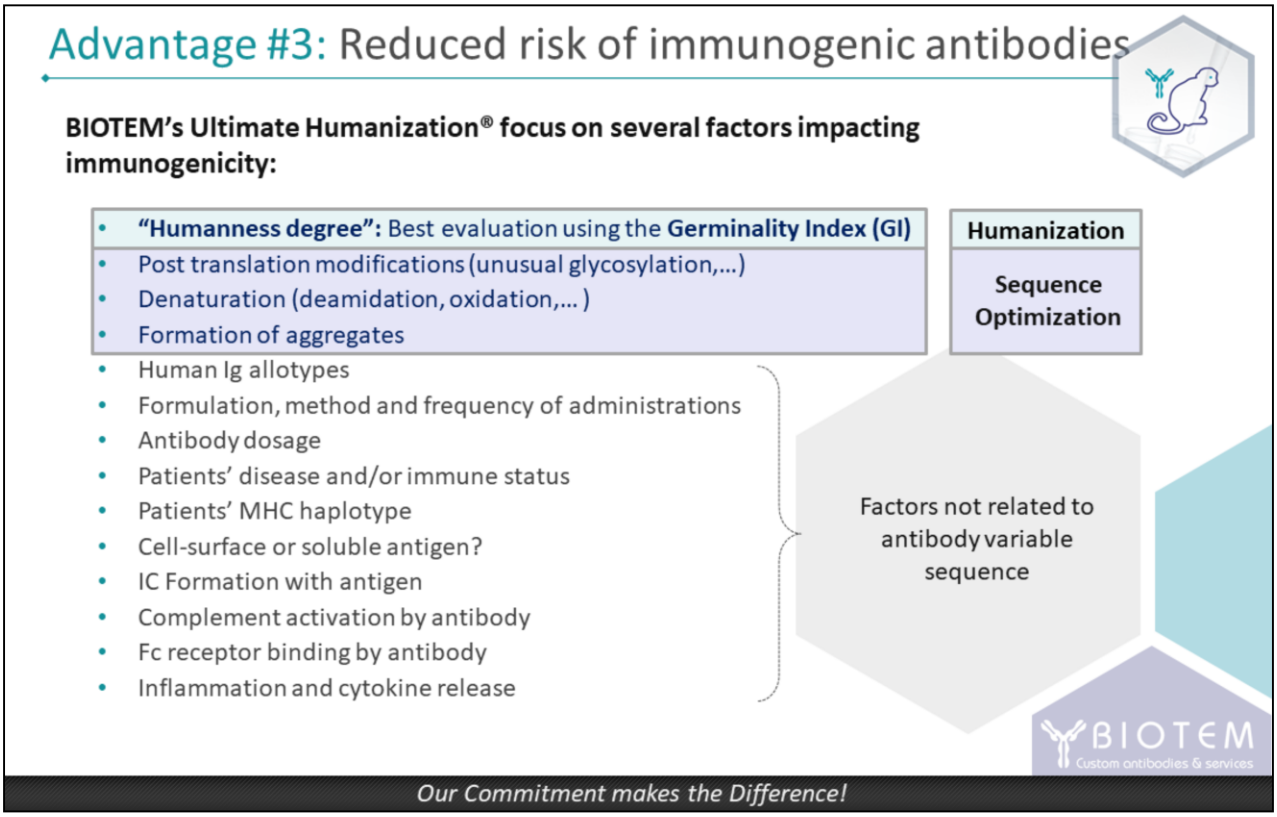


The 2nd advantage is related to the "off target" issue.

When a macaque generates antibodies against a given target (here in green a therapeutic target), there are natural safeguard mechanisms that will prevent the macaque to generate antibodies against its own antigens. The point is that macaques and humans have very similar proteomes with about 95 % identity!

As a results taking advantage on this high identity not only the macaque prevents the generation of antibodies to its own proteins but also in large part to the non-therapeutic human proteins.

In other words the active immunization of macaques is a simple strategy to naturally minimize off target issues very early in the development.



The 3rd and last advantage is related to the immunogenicity topic

Developing therapeutic antibodies with minimal immunogenicity is essential for efficacy and safety.

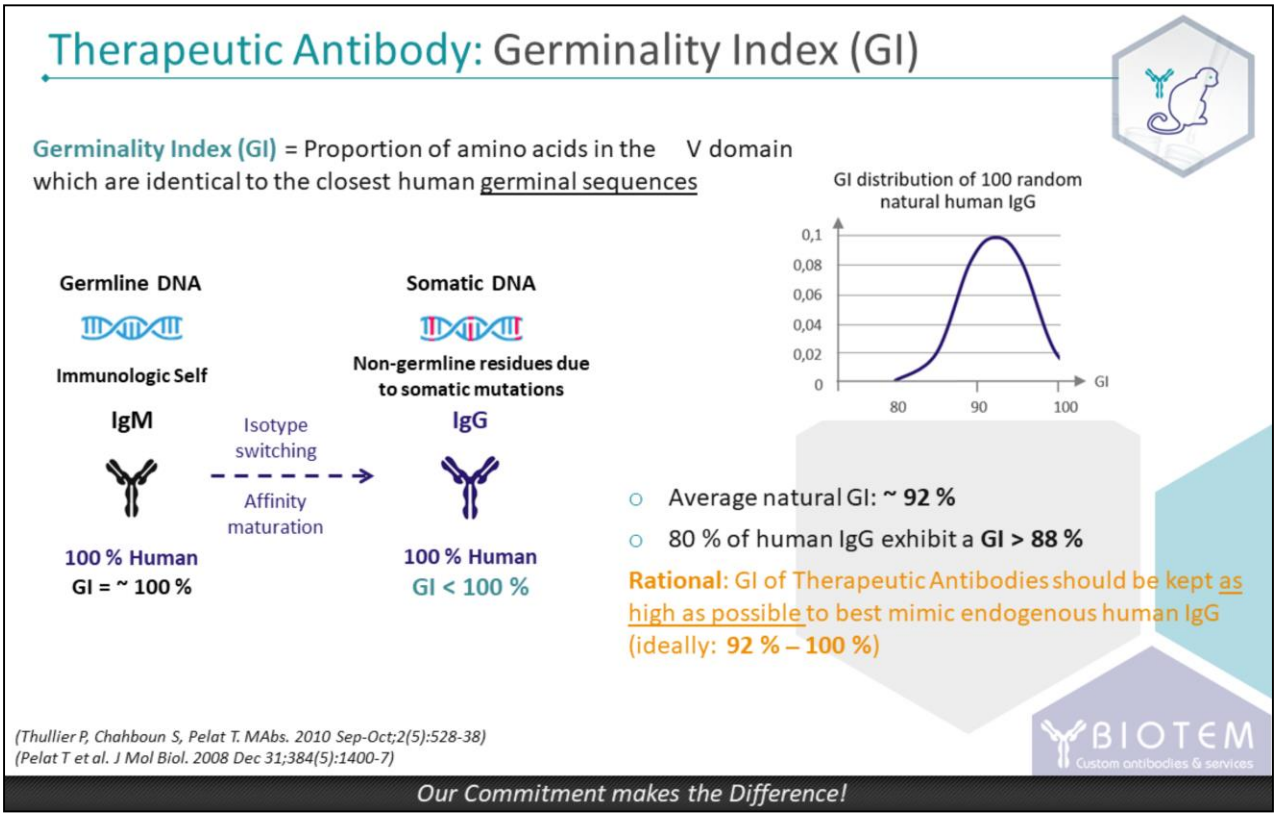
However immunogenicity can be modulated by numerous factors.

Those factors can be divided in two categories. In the first category you have factors related to the sequence of the antibody (and most particularly to the sequence of the variable regions).

Typically it is very easy to have access to antibody sequences early in the development, thus it should be evident that a relevant strategy would be to select or modify optimal candidate based on their optimal primary sequence.

Among the most impacting factors is of course the "humanness degree" with the assumption that the more human, the better.

At BIOTEM our gold standard is to use the **Germinality Index** in order to quantify the humanness degree of antibodies.



This **Germinality Index** has been first described and published by Thibaut PELAT in 2008. Thibaut is today working at BIOTEM and he is the head of the Recombinant & Therapeutic Antibody Department.

This index calculates for any antibody the proportion of amino acids in the variable domains (heavy and light) which are identical to the closest human germinal sequences.

Germline DNA is the source of DNA for all cells in the body and encodes for the true immunological self. As a result, IgM have GIs of 100 % because there are fully encoded by germline DNA without any mutations and should therefore be perfectly tolerated from a pure sequence view point. Indeed as we have seen there are many other factors, in particular those not related to the sequence that can impact immunogenicity.

Now during the process of affinity maturation, non-germline residues are usually incorporated into the variable regions of antibodies by somatic mutations. Consequently, the GI is reduced.

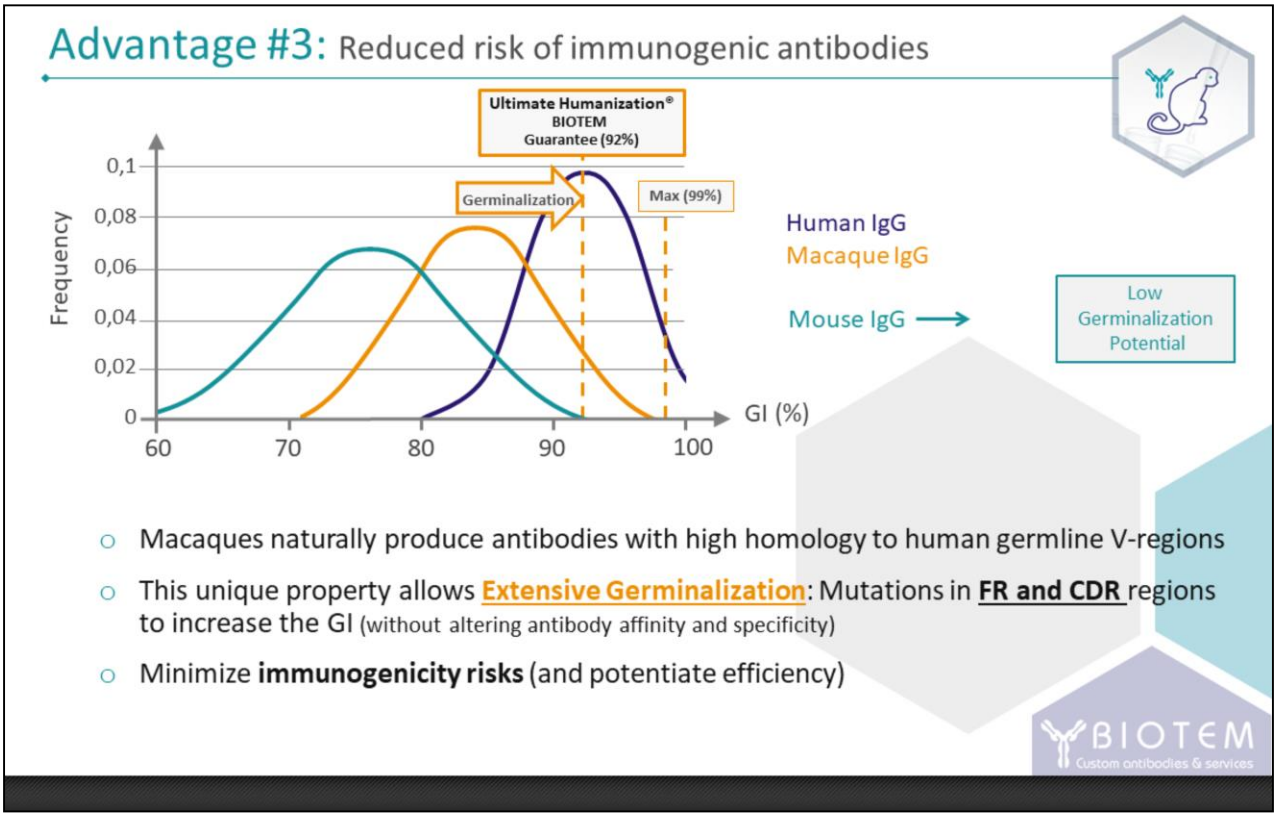
It is now well documented that those somatic mutations can generate T-cell epitopes and trigger immunogenicity.

If you analyze the sequence of many human IgG and calculate the GI frequency you will obtain a 'bell' curve.

We have determined that the average GI for natural human IgG is 92 % and that most human antibodies have a GI above 88 %.

Therefore our rational is to develop therapeutic antibodies with the highest GI possible in order to best mimic endogenous human IgG, minimize potential T cell epitope occurrence and at the end minimize immunogenicity issue.

And this is where macaques give us a great help!



It turns out that macaques naturally produce antibodies with very high homology to human germline variable regions with naturally high GI, **on average 85 %.**

This unique property allows to perform a process that we call **Extensive Germinalization**.

During this process amino acid substitutions in the FR and even in the CDR regions are introduced in order to increase further the GI to a minimum of 92 % and up to 99 %. Of course only mutations that do not affect affinity and specificity are allowed.

Thus the 3rd advantage of our Ultimate Humanization technology is to generate germinalized antibodies with extremely high GI which minimize immunogenicity risks.

Just for comparison, mice naturally produce IgG with much lower GI (on average 75 %) making such extensive germinalization process impossible in most case.

Now we will briefly show a case study to exemplify this germinalization process. We decided to develop therapeutic antibodies against a well known target: TNF-α.

Case Study

State-of-the-Art
Anti-TNF-α
Development

❖ **An example of what can be obtained in a 9 – 13 month project!**

Our Commitment makes the Difference!

TNF-α: Why this choice?

Table of Anti-TNF-α Therapeutic Antibodies, approved

Modified from https://en.wikipedia.org/wiki/List_of_therapeutic_monoclonal_antibodies (last consulted on Oct. 23, 2020)

INN name	Trade name	Type	Humaness	Comment	Approved	Use
infliximab	Remicade®	mab	Chimeric, partially humanized	IgG, human-mouse monoclonal cA2 heavy chain, disulfide with human-mouse monoclonal cA2 light chain	FDA approv. in 1998, EU approv. in 1999	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis
infliximab (BIOSIMILAR)	Remsima®	mab	idem above	idem above	EU approv. in Sept. 2013 as a biosimilar	idem above
adalimumab	Humira®	mab	human	IgG1, human monoclonal D2E7 heavy chain, disulfide with human monoclonal D2E7 light chain	FDA approv. in 2002, EU approv. in Sept. 2003	Rheumatoid arthritis, Crohn's disease, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, hemolytic disease of the newborn
certolizumab pegol	Cimzia®	Fab'	humanized	Fab' fragment, human-mouse monoclonal CDP870 heavy chain, disulfide with human-mouse monoclonal CDP870 light chain, pegylated at Cys-221	FDA approv. in April 2008, EU approv. in Oct. 2009	Crohn's disease, rheumatoid arthritis, axial spondyloarthritis, psoriasis arthritis
golimumab	Simponi®	mab	human	IgG1, human monoclonal CNTO 148 g1-chain, disulfide with human monoclonal CNTO 148 kchain	FDA approv. in April 2009, EU approv. in Oct. 2009	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis

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Why did we choose anti-TNF-α therapeutic antibodies as there are already so many on the market ?

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

Antibody INN (trade name)	Target	Strategy	ADA	GI
ramucirumab, 1121B, IMC-1121B (Cyramza®)	VEGF R2	Human (Naïve Library)	None Described	92%
briakinumab (ABT-874)	IL12/IL23		Unknown	90%
figitumumab, CP-751871	IGF1R	Human (Tg mice)	Unknown	96%
adalimumab (Humira®)	**TNF-α**		**None Described**	**95%**
ustekinumab (Stelara®)	IL12/IL23		YES	92%
canakinumab (Ilaris®)	IL 1		None Described	83%
tocilizumab (Actemra®)	IL 6R	Humanized	YES	88%
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, rhuMAB 2C4 (Omnitarg®)	ERBB2 (HER2)		None Described	84%
alemtuzumab (CAMPATH-1H, MABCAMPATH®)	CD52		YES	82%
farletuzumab, M3, MORAb-003	FOLR1		Unknown	80%
teplizumab, humanized OKT3®	CD3		YES	79%
infliximab (Remicade®)	**TNF-α**		**YES**	**71%**

- Average GI for **humanized antibodies** is substantially low (~84%) with a high proportion of ADA
- Large GI amplitude for **fully human antibodies** (83%-96%)

General deleterious effects often linked to lower GIs

The point is that even for many approved therapeutic antibodies there are still efficacy and safety issues and in particular for anti-TNF antibodies (in red in the chart).

Indeed the table presents a non exhaustive and short list of approved therapeutic antibodies with described side effects.

Not surprisingly it is possible to correlate a low GI with the presence of ADAs.

Side effects might also be attributed to off-target binging as well as the result of indirect therapeutic mechanism.

As far as TNF antibodies are concerned it is very common to change the administered molecule during the treatments to counteract the loss of therapeutic efficacy of one given therapeutic antibody.

As a result there is still a need for new, safer anti-TNF-α therapeutic antibodies.

TNF-α: Many Biobetters in Development – Few examples

INN Name	Type	Humaness	Status	Comment
afelimomab	$F(ab')_2$	mouse origin	Discontinued	Administration of afelimomab reduces the concentration of interleukin-6 in patients with sepsis, but reduces mortality only marginally
nerelimomab	mab	mouse origin		IgG1, mouse monoclonal BAYX1351 γ1-chain anti-human TNF-α, disulfide with mouse monoclonal BAYX1351 light chain
ozoralizumab	nanobody	humanized		Ozoralizumab (ATN-103), a trivalent albumin-binding nanobody that neutralizes TNF-α
placulumab	mab	human	Discontinued	This drug was developed by Teva Pharmaceutical Industries, Inc. As of 2012, development of placulumab has been discontinued

❖ **Projects with variable outputs** depending on many parameters

Our Commitment makes the Difference!

Everyone would agree and, indeed, there are many many Anti-TNF biobetters in development!

A webinar - 2 years ago by Rakesh Dixit from Medimmune - mentioned that there were 15 biobetters in development for anti-TNF antibodies at that time!

7 biobetters relative to adalimumab

8 biobetters relative to infliximab

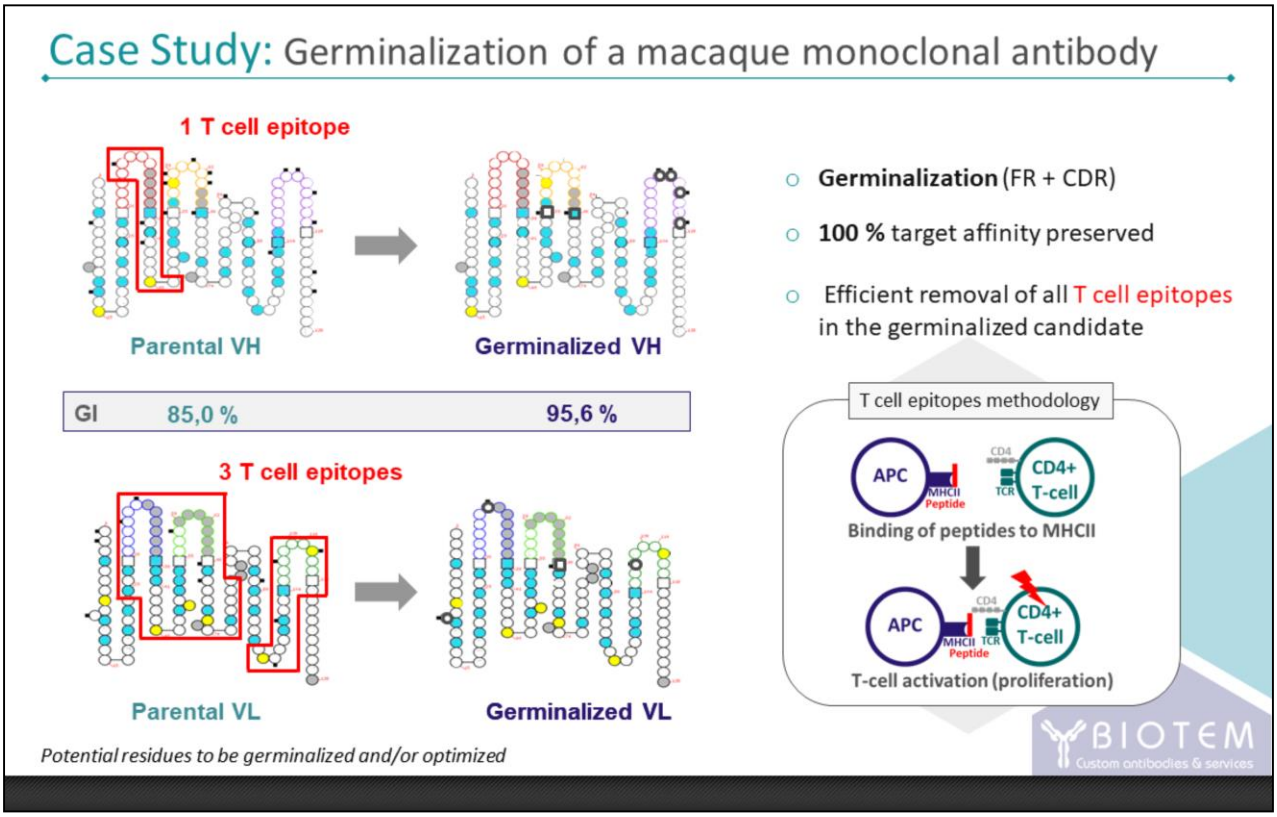
So we decided it would be interesting to compare the results of our technology to these efforts.

Also considered in our aim to demonstrate the strength of our technology, it is important to note that human TNF-α is 97% identical to the macaque orthologue which would make it an ideal candidate to showcase the species-barrier lifting techniques efficacy.

For all these reasons we chose TNF-α to demonstrate the efficacy of our Ultimate Humanization technology.

After immunizing 1 macaque with a recombinant TNF-α we did obtain many unique high affinity antibodies with a large epitope coverage.

We will now show the germinalization process of 1 of these clones



For those who are not familiar this is a representation of heavy and light variable regions of the parental macaque antibodies. Each circle represents 1 amino acid and black dots indicate differences with regards to the closest human germinal sequences.

We calculated that the parental macaque antibody has a GI of 85 % (which is also the average GI for macaque antibodies).

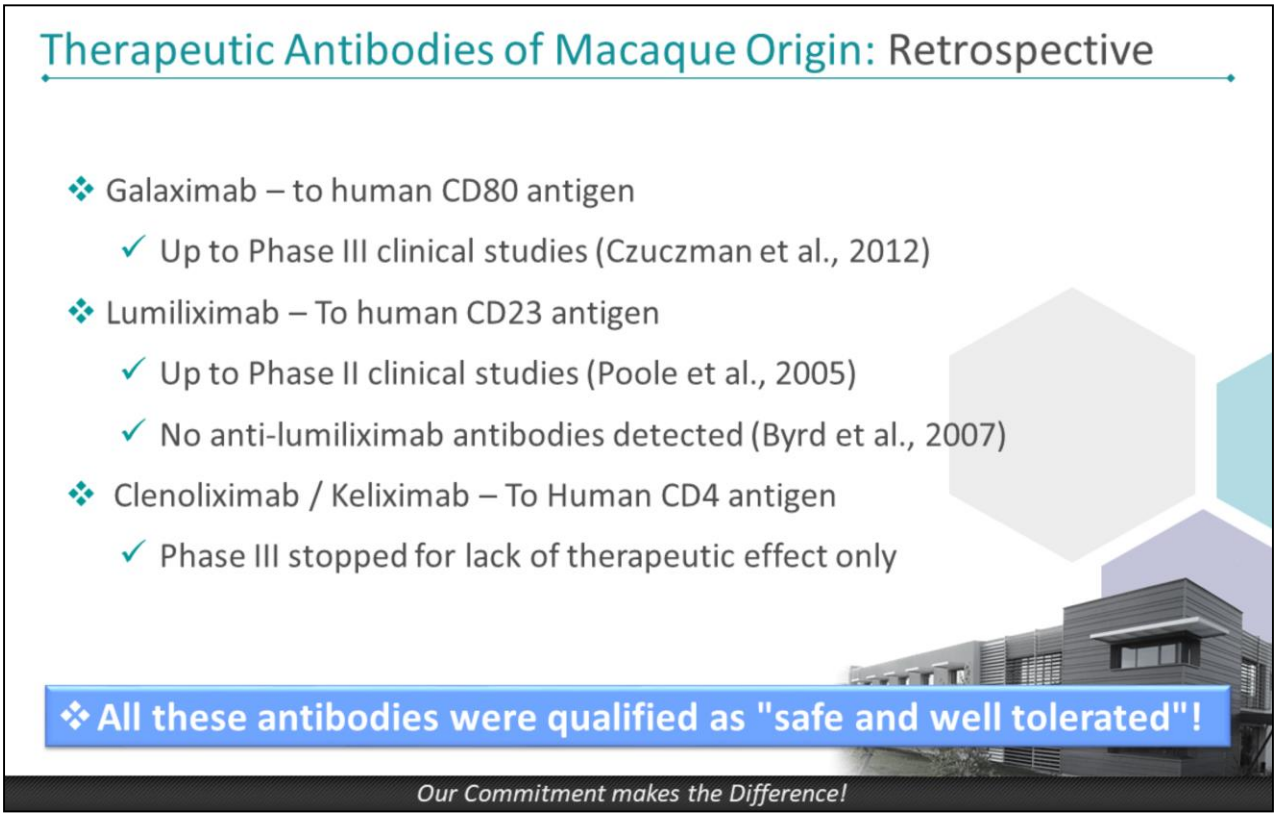
During the germinalization most divergent amino acid could be mutated to the human germline sequences except those indicated by bold circles. Those residues where identify for being important in order to preserve the affinity.

After the complete process the germinalized antibody exhibits a GI of 95,6 % which is much better than the average GI for human antibodies. Most none essential modifications naturally generated by the macaque where reverted during the process.

In a second part of the work we wanted to verify if the germinalized antibody would be less immunogenic.

Indeed using an *ex vivo* approved methodology we were able to show that all T cell epitopes responsible for immunogenicity had been removed in the germinalized candidate.

It is not possible to show here more confidential results regarding the overall safety of antibodies from macaques but a number of such molecules were tested <u>up to Phase III clinical studies</u>

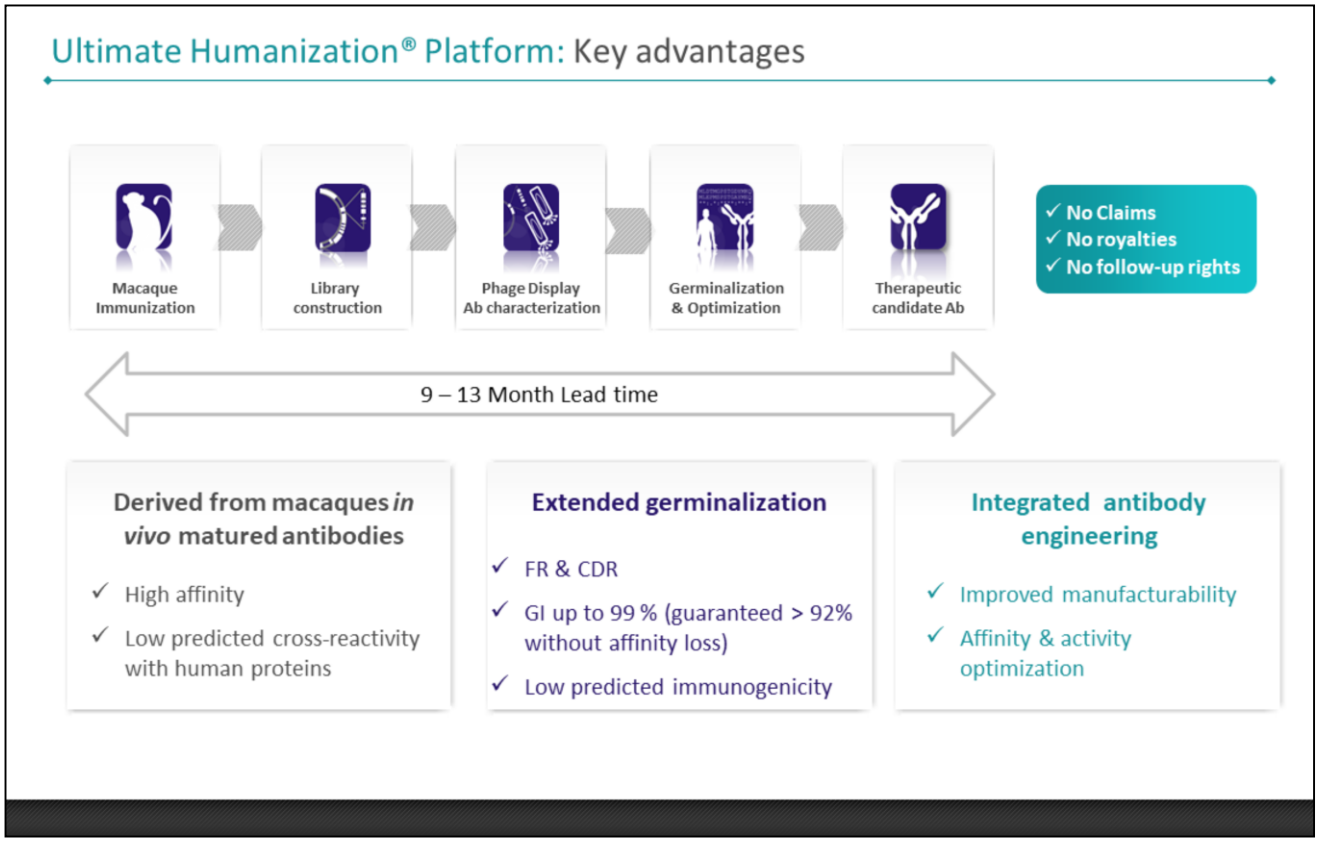


Indeed the three mabs (and the attached scientific literature) listed here describe the behavior of these therapeutic antibodies with various mechanisms of action.

Some lack of therapeutic effects have been unfortunately reported.

But no contraindication could be noted and no safety issues linked to the presence of ADA wetre reported.

All these antibodies were qualified as "safe and well tolerated"!



In conclusion, summarizing the 3 main advantages of our Ultimate Humanization technology:

1. It is easily achievable to obtain high affinity antibodies to virtually any target by conventional immunization of macaques
2. The obtained antibodies are naturally screened *in vivo* by the macaque immune tolerance mechanism, to remove many potential clones likely to cross react with non-therapeutic target human proteins thus minimizing off target issues
3. The antibodies have natural high GI which make them compatible with an extended germinalization process in order to remove most T cell epitopes, thus minimizing immunogenicity and the generation of ADAs

All together the Ultimate Humanization technology allows to rapidly (in about 1 year) and efficiently generate potential therapeutic antibodies with intrinsic high properties in order to increase safety and efficacy.


BIOTEM
Custom Antibodies & Services
info@biotem.fr
+33 (0)4 76 65 10 91
www.biotem-antibody.com