



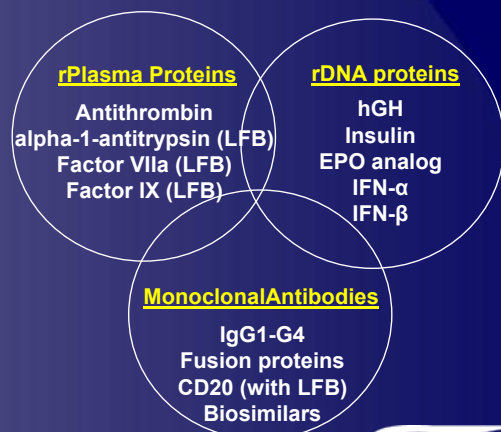
Transgenic Production:

A validated, low-cost, highly scaleable solution for the production of difficult-to-express and high volume therapeutic proteins

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- Public biotechnology company
 - Based in Framingham, MA (USA)
 - >150 Employees
- Leader in production of therapeutic proteins in milk
- ATryn® (recombinant human antithrombin)
 - lead product approved by EMEA
 - BLA filed with FDA July 2008; Fast Track/Orphan Drug status; approval anticipated Q1/2009
- Product-based partnerships with Leo Pharma, Ovation and LFB Biotechnologies

Significant scientific, manufacturing & regulatory experience in developing biologics (>100 different proteins)



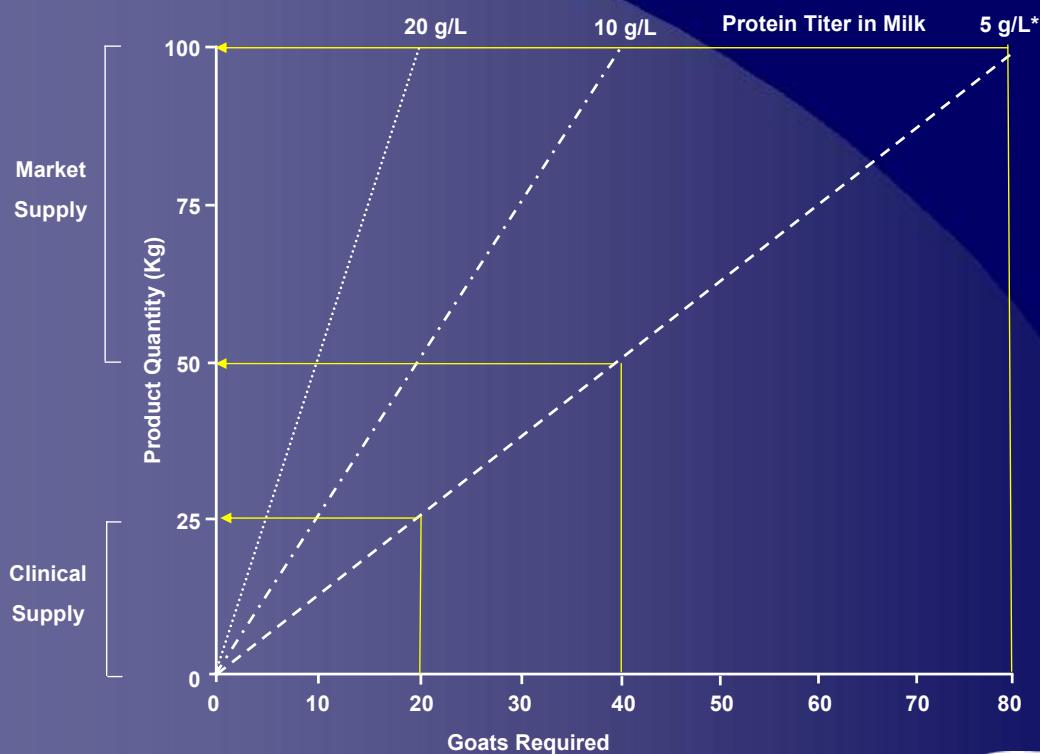
Strategic Advantages of Transgenics

- Transgenic production technology offers;
 - Comparable lead times vs. cell culture
 - Efficient production of difficult-to-express proteins
 - E.g. Antithrombin III, Factor VIIa and Factor IX
 - Competitive COGS vs. leading edge cell culture methods
 - Significantly reduced upfront capital expense
 - Easy scale-up (10 → 1000s kgs)
 - E.g. Alpha-1-Antitrypsin (>1 metric tonne), Mabs (100+ Kg)
 - Validated regulatory pathway
 - IP protection and simplified IP requirements
 - Enhanced ADCC

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Scalability of transgenic platform



* Average transgenic yield of MAb (figures assume 500L milk/goat/year and 50% process yield)

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Significantly lower capital expenditures



78,125 – 156,250 L Cell Culture Facility*



1,500 Goat Milk Production Facility*

(Annual Output 3,750 – 7,500 Kg)

Mean CapEx = \$550M – \$1,100M (includes DSP)

Cell Culture CapEx @ 35% = **\$200M – \$400M**

Actual Investment to date = **\$25M**

* Assumes 2 g/L productivity

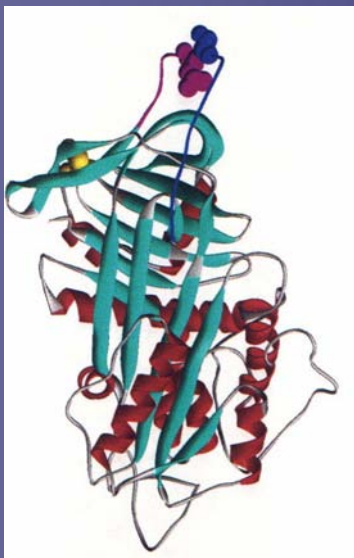
* Based on actual productivity (5 g/L and 10g/L)

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Difficult to Express Proteins

ATryn[®]: Recombinant Human Antithrombin

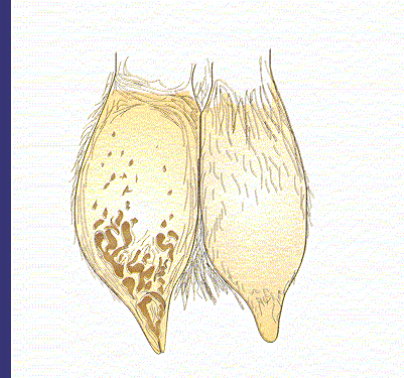


- Difficult-to-Express in Cell Culture
- Anticoagulation and Anti-inflammatory Properties
 - Large therapeutic potential
- Hereditary Deficiency (HD)
 - Initial approval validation
- Acquired Deficiencies (AD)
 - US DIC Market \$2-3 Billion
 - US HR Market \$100-150 Million

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Scaleable, Efficient & Cost-Competitive Production System



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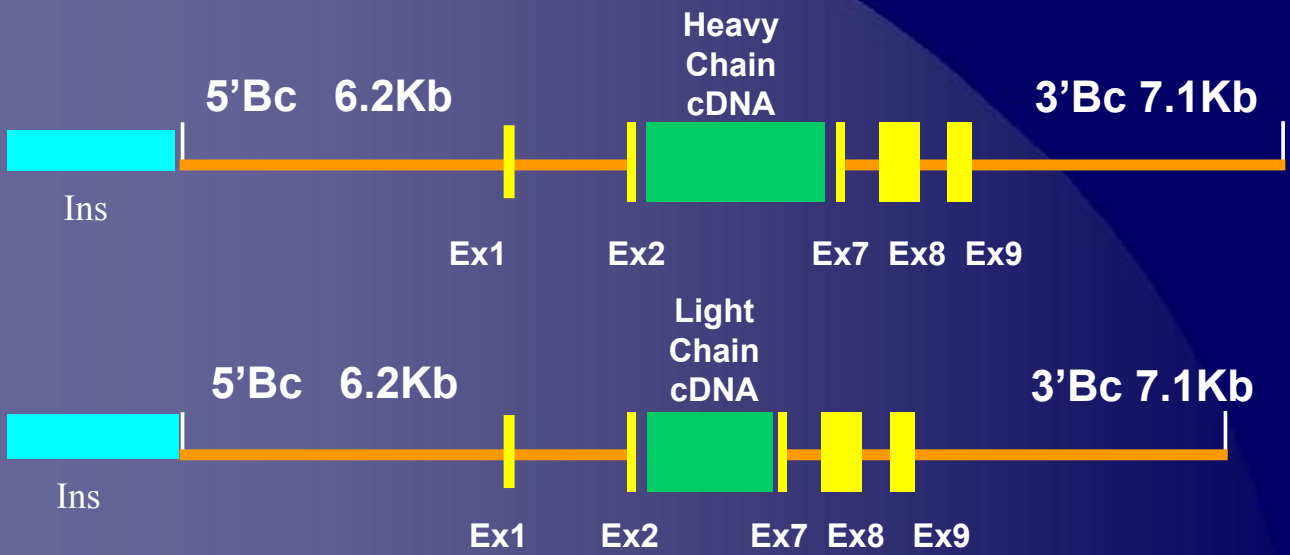
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Mammary Gland

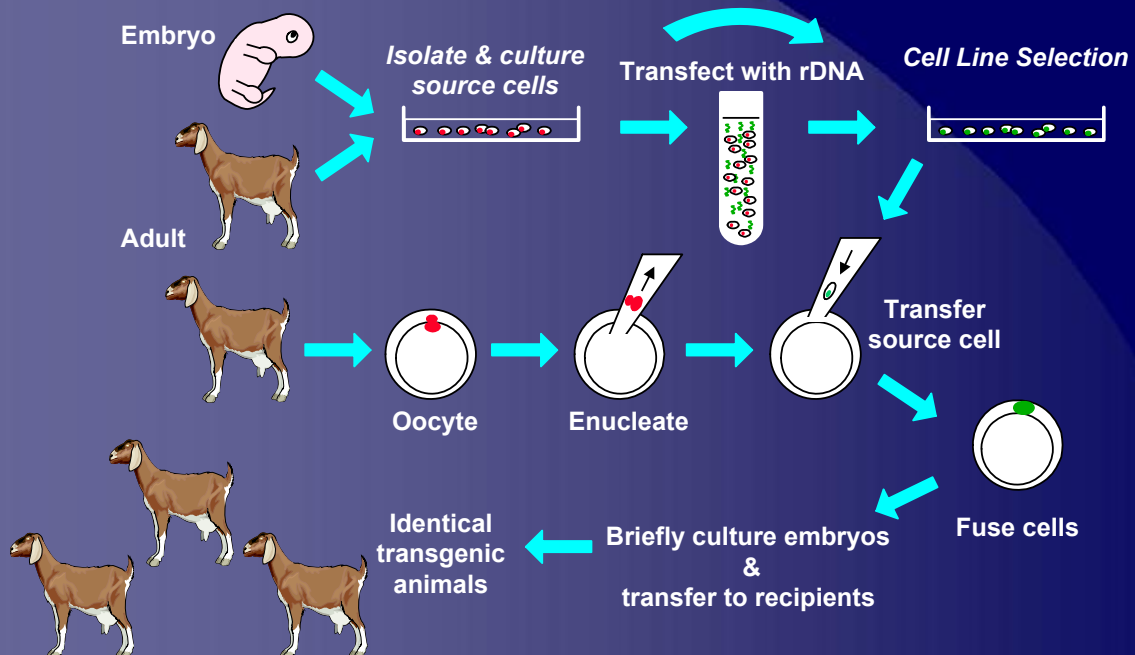
- Major function is to secrete proteins
- Post translational modifications:
 - glycosylation, gamma carboxylation
- High concentration of cells
 - 100 to 1,000 fold higher than tissue culture
- Inducible system
- Major proteins found in milk at 40 mg/ml

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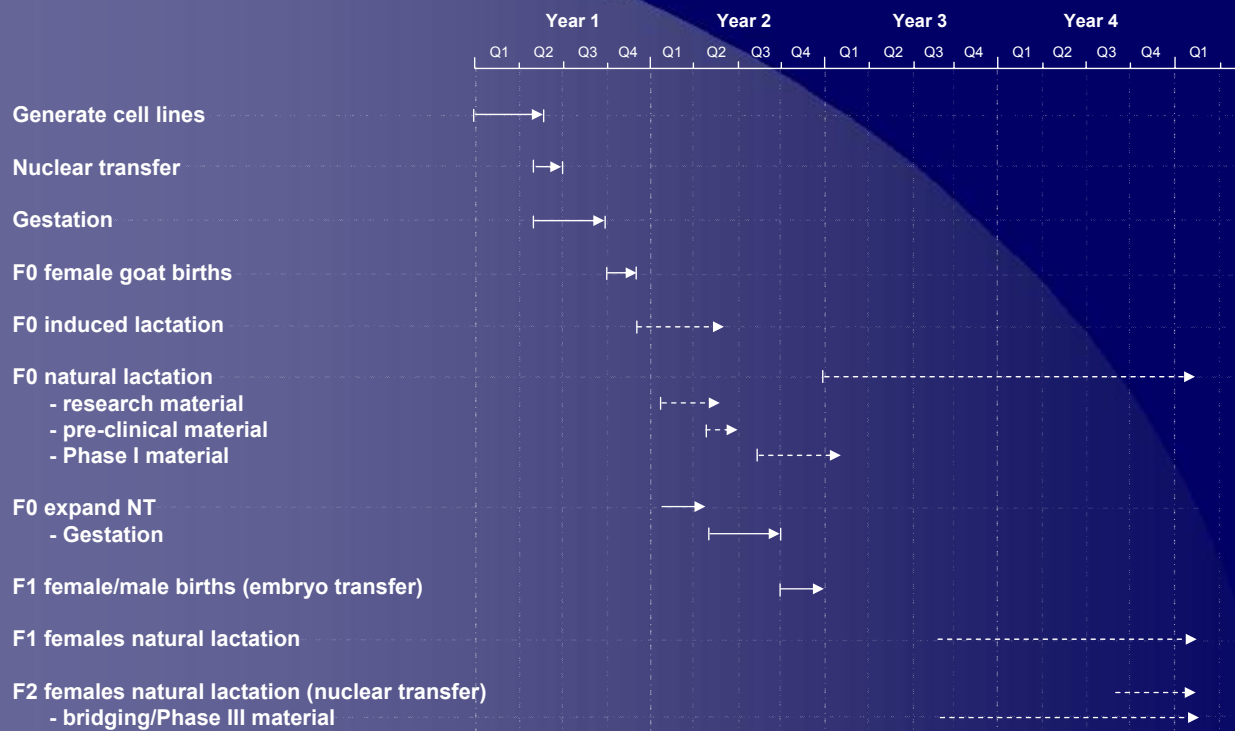
Expression Cassettes for Transgenic Monoclonal Antibodies in Milk



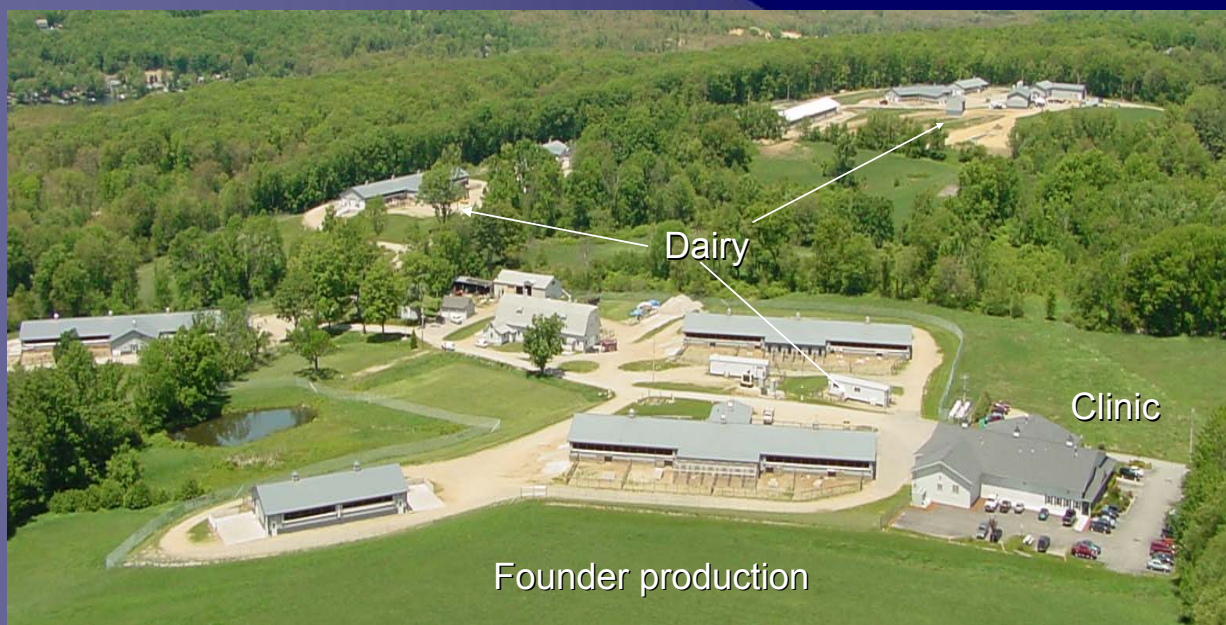
Nuclear Transfer for Production of Founder Transgenic Goats



Timeline for development of Transgenic Goat



GTC Production Site – Massachusetts, USA



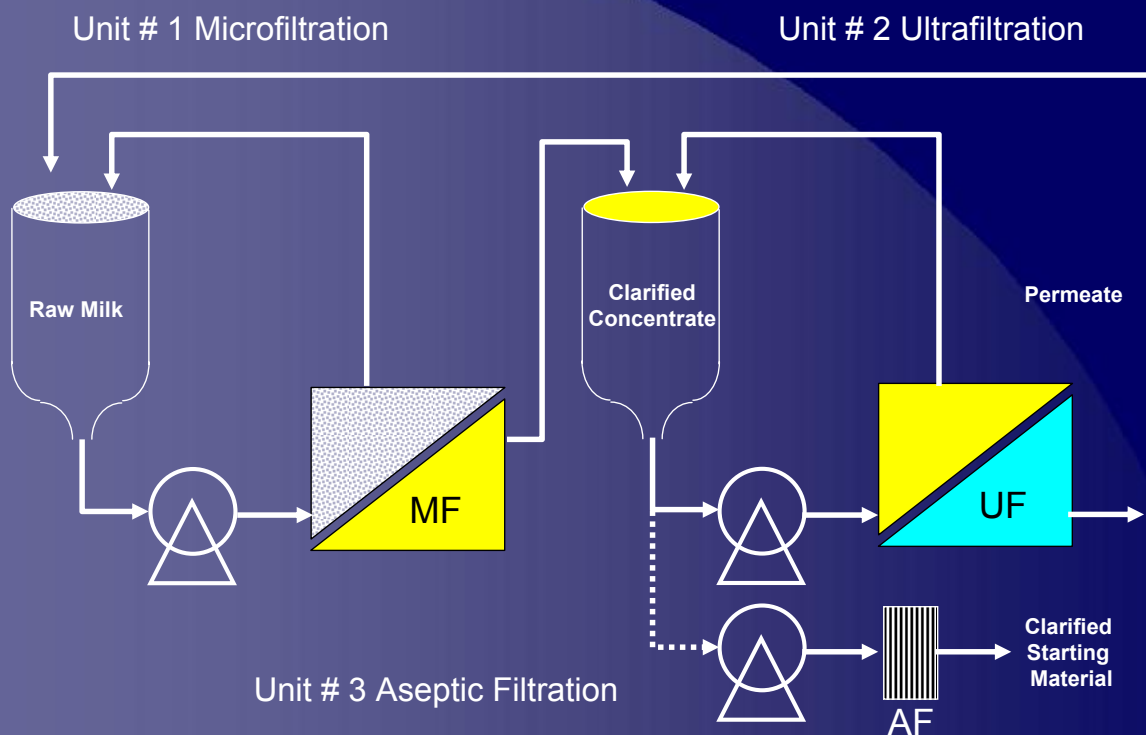
Source Material Collection



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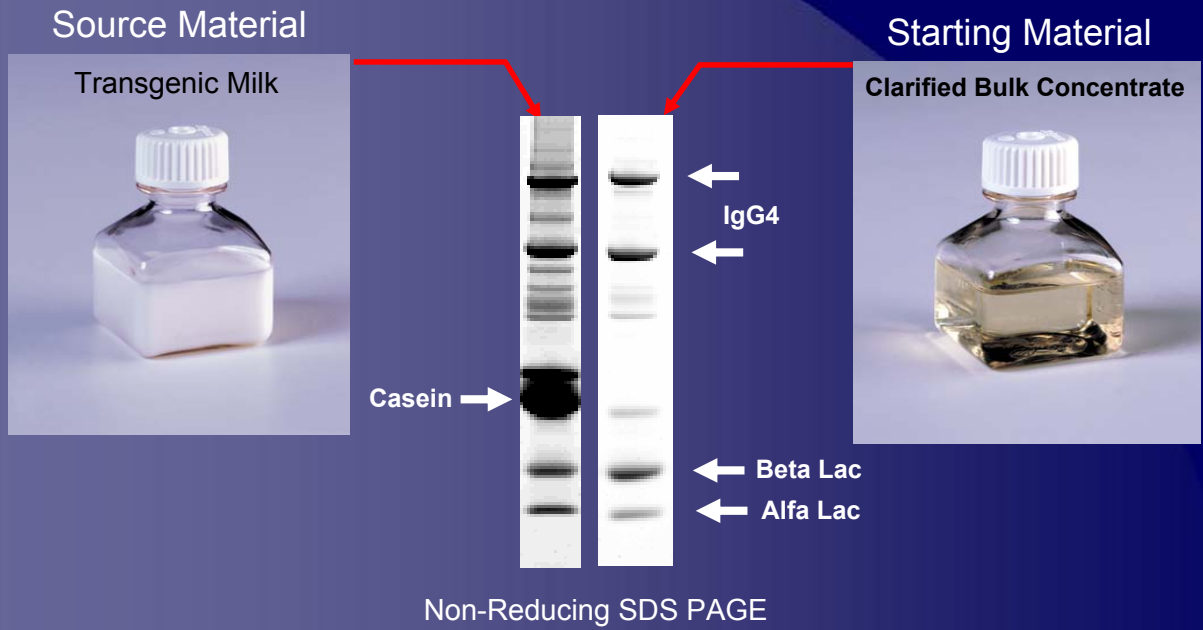
Clarification Process Schematic



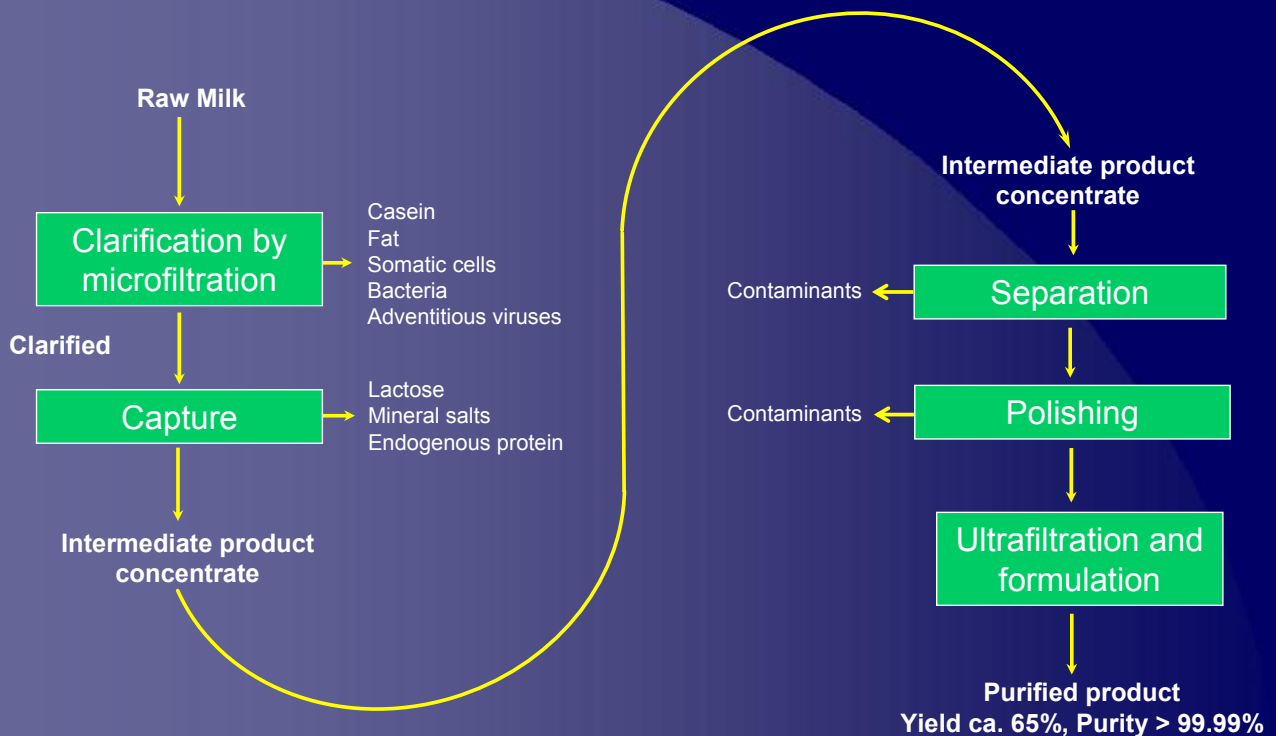
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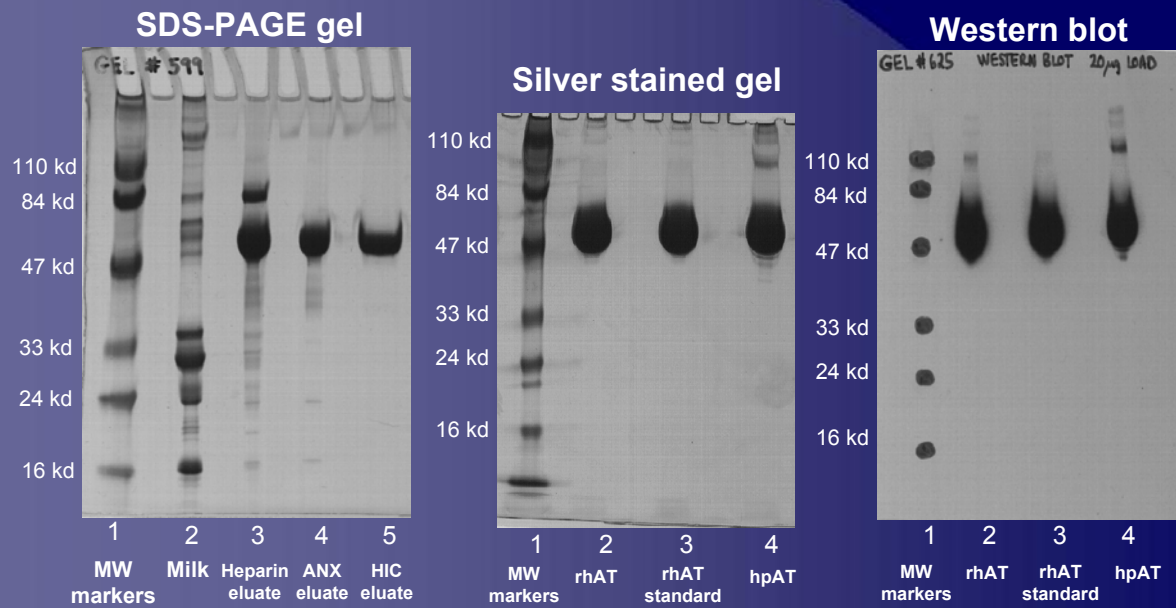
Efficient Milk Clarification Process



DSP of Transgenic Proteins from Milk



Purity profile for ATryn® (process & product)



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Impurity Considerations

- Goat milk proteins
- Residual heparin
- Viruses and other adventitious agents
- Prions

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Routine QC Tests

Source Material

- Bioburden
- Endotoxin by LAL
- Viral
- Concentration
- Activity

Drug Substance

- Osmolality
- pH
- Bioburden
- Mycoplasma
- Endotoxin by LAL
- Residual Milk Proteins (ELISA)
- Concentration
- Activity
- Purity by SDS-PAGE
- Residual DNA
- Peptide Map
- Aggregation by HPSE
- Glycan Profiling
- Purity by RP-HPLC

Drug Product

- Activity
- Aggregation by HPSEC
- Bacterial Endotoxin
- General Safety
- Identity ELISA
- Moisture
- Osmolality
- Sterility
- Bioburden
- Concentration
- Oxygen headspace
- Particulates
- SDS Page Purity
- RP HPLC Purity
- pH

Impurity Profile

Impurity	ppm
Goat AT LiCor Western (LOD is 2.5 ng/mg)	≤ 2.5*
Soluble goat milk proteins ELISA (LOQ is 0.2 ppm) (detects primarily α-lactalbumin, albumin, β-lactoglobulin)	0.4 to 1
Colloidal goat milk proteins ELISA (LOQ is 0.9 ppm) (detects primarily α-s1-casein, α-s2-casein, β-casein and κ-casein)	≤ 0.9
Goat IgG ELISA (LOQ is 0.3 ppm) (detects primarily goat IgG with some cross-reactivity for IgA & IgM)	≤ 0.3
Immunological Detection of Contaminating Proteins ELISA (LOQ is 0.3 ppm) (detects primarily lactoferrin & lactoperoxidase)	≤ 0.3
Total maximum possible impurities	≤ 5 (3.7*)

*Based on the process validation log reduction, this number for goat AT is actually ≤ 1.2 ppm

Adventitious Agent Minimization

- **Goat**
 - Animal selection (mainly closed NZ herd)
 - Periodic testing & superior husbandry with risk minimization measures (Good Agricultural Practices)
 - Closed USDA certified scrapie free herd
- **Milk (Source/Starting Material)**
 - *In vitro* cell line screen for viruses
 - Testing for other adventitious agents including zoonotic pathogens and antibiotic residues
- **Purification Process**
 - Validation for DNA, viral and prion removal capacity
 - Tested for endotoxin, contaminating protein & residual-heparin removal capability

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Viral Considerations

- hAT goats are tested and are free of viruses of concern.
- Milk is screened for unknown viruses and all have been negative to date.
- Manufacturing process validated to remove $\geq 16 \log_{10}$ to $\geq 25 \log_{10}$ of model viruses.

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Prion Considerations

- The prion-disease that can affect goats is scrapie.
- rhAT containing milk is collected from Certified Scrapie-free goats in a “negligible TSE-risk herd”.
- Milk (and semen) are considered non-infectious for scrapie & classified category IV by WHO (no detectable infectivity).
- The rhAT purification process can remove $\geq 11 \log_{10}$ of scrapie prions.

Glycosylation: different, but unlikely to be clinically relevant

- No sign of immunogenicity of ATryn to date – suggests goat-derived sugars are not inherently immunogenic
- All sugars on goat-derived proteins are also found on human or CHO-derived proteins (i.e. there are no sugars unique to goats)
- Differences in glycosylation patterns between goat and CHO-derived proteins are simply due to relative amounts of sugars (i.e. not in the identity of the sugars)
- No evidence of gal- α -1,3-gal on ATryn (NS0-derived Erbitux)

ATryn Safety Demonstrated

- Contaminating proteins \leq 5 ppm
- Free of adventitious agents, including viruses and prions
- Non-immunogenic in human clinical trials

High Volume Protein Production



Projected Material Requirements for Key MAbs

Demand (kg)	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
ENBREL	580	599	619	640	661	683	706	718	730	742
REMICADE	536	569	605	643	684	727	772	796	822	847
HERCEPTIN	453	483	515	550	587	626	668	690	714	738
HUMIRA	108	127	150	177	208	245	288	314	342	372
AVASTIN	86	105	129	158	194	237	290	323	360	400
Total (kg)	1763	1883	2018	2168	2334	2518	2724	2841	2968	3099

Wide Variety of Monoclonal Antibodies and Antibody Fusions Successfully Expressed

- Monoclonal Abs
 - IgG1 chimeric
 - IgG1 humanized
 - IgG1 human
 - IgG2
 - IgG4
 - IgG4 mutant
 - IgG2/4
 - IgG3 mouse
- MAb fusions
 - IgG1-fusion CTLA4
 - IgG1-fusion CTLA4*
 - IgG4-fusion to CTLA4
 - CD4-IgG2
 - IgG1-glucuronidase
 - IgG1-angiogenin
 - IgG1-carboxypeptidase
- Single chain antibody

High Expression Levels of Monoclonal Antibodies in Transgenic Goat Milk

Antibody	Target	Expression Levels
human	Colon cancer	1-3 g/L
humanized	Lewis Y	up to 5 g/L
human	TNF-alpha	up to 5 g/L
murine	TNF-alpha	2 g/L
chimeric	TNF-alpha	5 g/L
human	IL-8	up to 7 g/L
humanized	Alpha-4-integrin	6 g/L
humanized	Amyloid B	1 to 10 g/L
CD-137 chimeric	4-1BB	5 -15 g/L

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6 Goat Production System

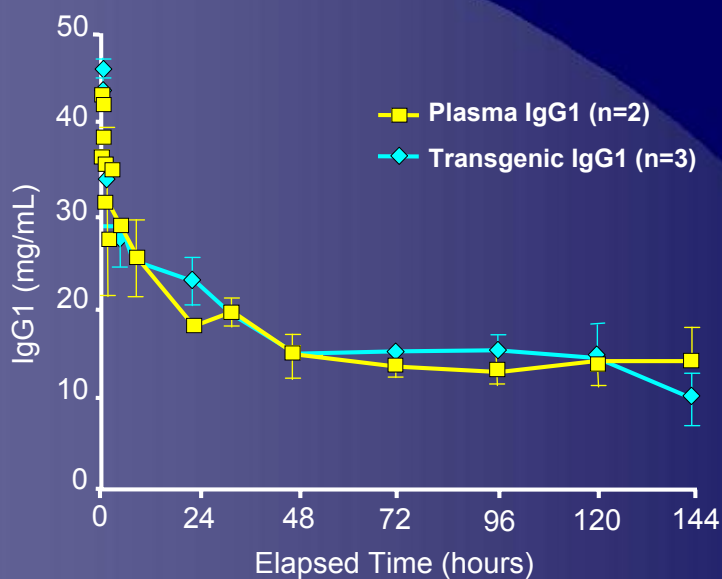


- 6 goats producing a humanized IgG4 MAbs
- Total IgG4 produced = 21.79 kg

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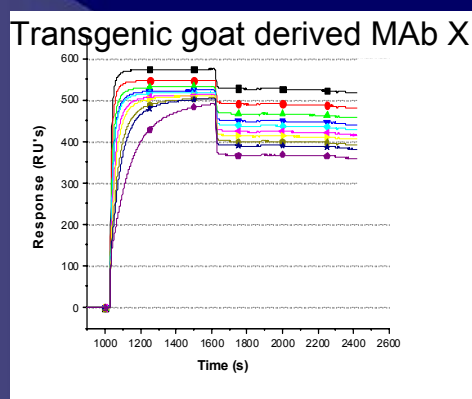
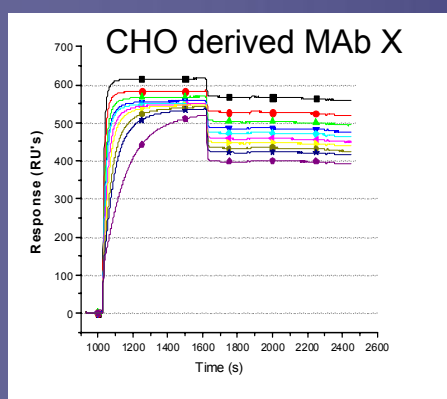
Equivalent Pharmacokinetics: Tg vs. Plasma IgG1



Comparison of pharmacokinetics - transgenic IgG1 vs. human, plasma-derived IgG1 in mice. Clearance of both antibodies is shown as the decrease in serum (% remaining) as a function of time after i.v. injection of 2 mg/kg of antibody.

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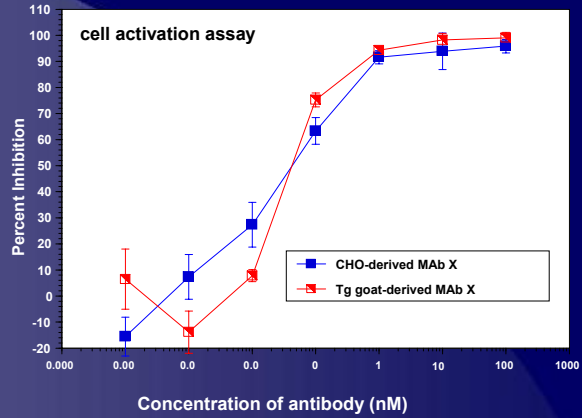
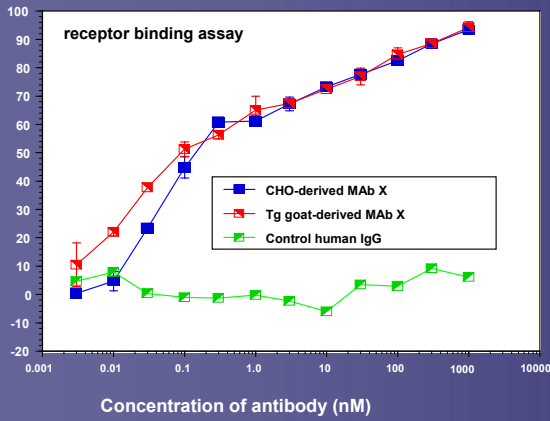
Transgenic antibodies have equivalent affinity to CHO



	<u>CHO</u>	<u>Transgenic</u>
K_d off-rate constant (s^{-1})	$4.84 \pm 2.10 \times 10^{-5}$	$4.60 \pm 1.71 \times 10^{-5}$
K_a on-rate constant (s^{-1})	$4.33 \pm 2.40 \times 10^{-5}$	$5.00 \pm 2.49 \times 10^{-5}$
Affinity (M^{-1})	0.90×10^{-10}	1.12×10^{-10}

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Transgenic antibodies equivalent to CHO in bioassays



Approximate IC_{50} values

CHO

Transgenic

Receptor binding assay (M^{-1})

1×10^{-10}

1×10^{-10}

Cell activation assay (M^{-1})

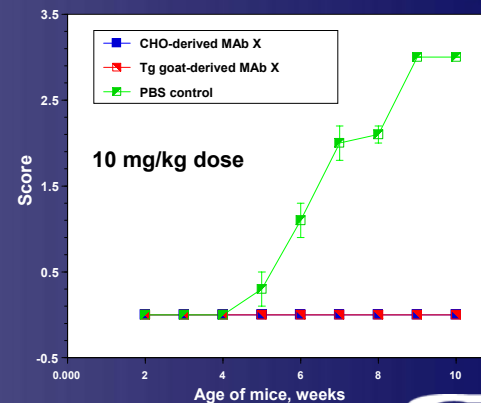
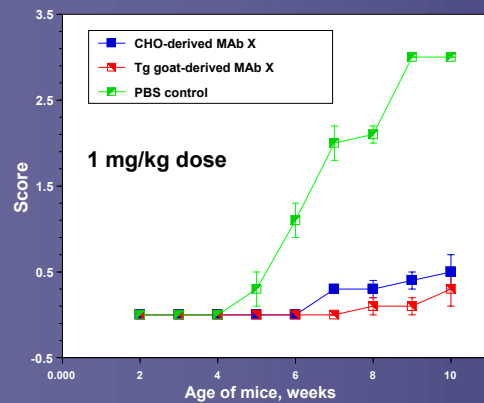
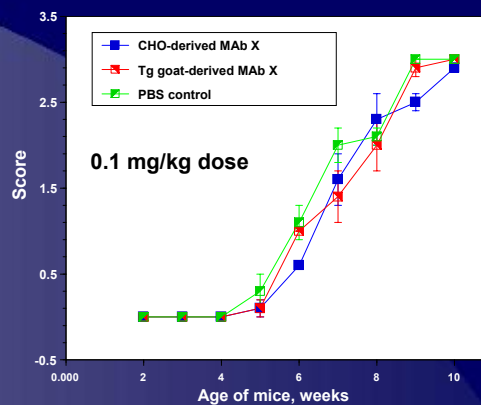
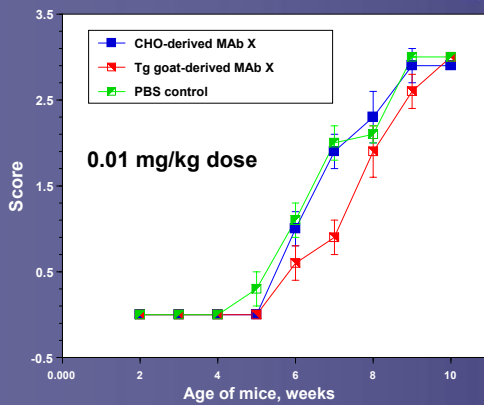
2×10^{-11}

3×10^{-11}

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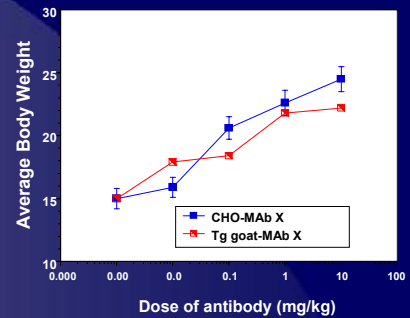
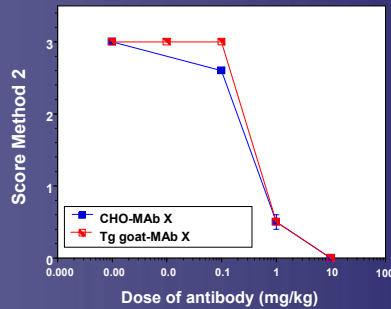
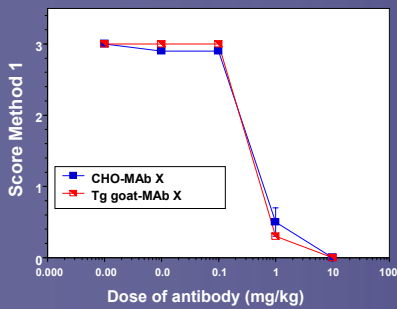
Equivalent efficacy in animal model



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Equivalent efficacy in animal model (week 10)



	ED ₅₀ (mg/kg)	
	<u>CHO</u>	<u>Transgenic</u>
Score Method 1	0.1 – 1	0.1 - 1
Score Method 2	0.1 – 1	0.1 - 1

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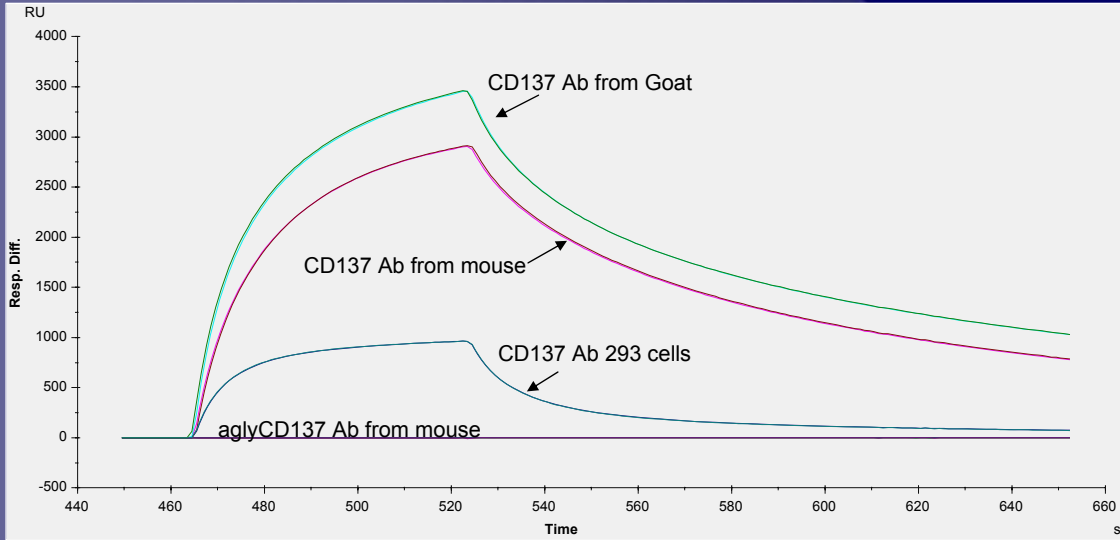
Transgenic MAbs have naturally enhanced ADCC

- Majority of milk-produced MAb → high mannose
 - Whereas usual tissue culture MAb → fucosylated complex
- High mannose MAb lacks fucose
- High mannose MAb IgG has same pK as tissue culture derived & plasma derived IgG
- Collaborated with Genzyme to study ADCC
- Established that milk produced CD137 MAb;
 - Carries high mannose
 - Binds more tightly to CD16
 - Is capable of killing tumor cell lines, whereas cell culture derived MAb is not

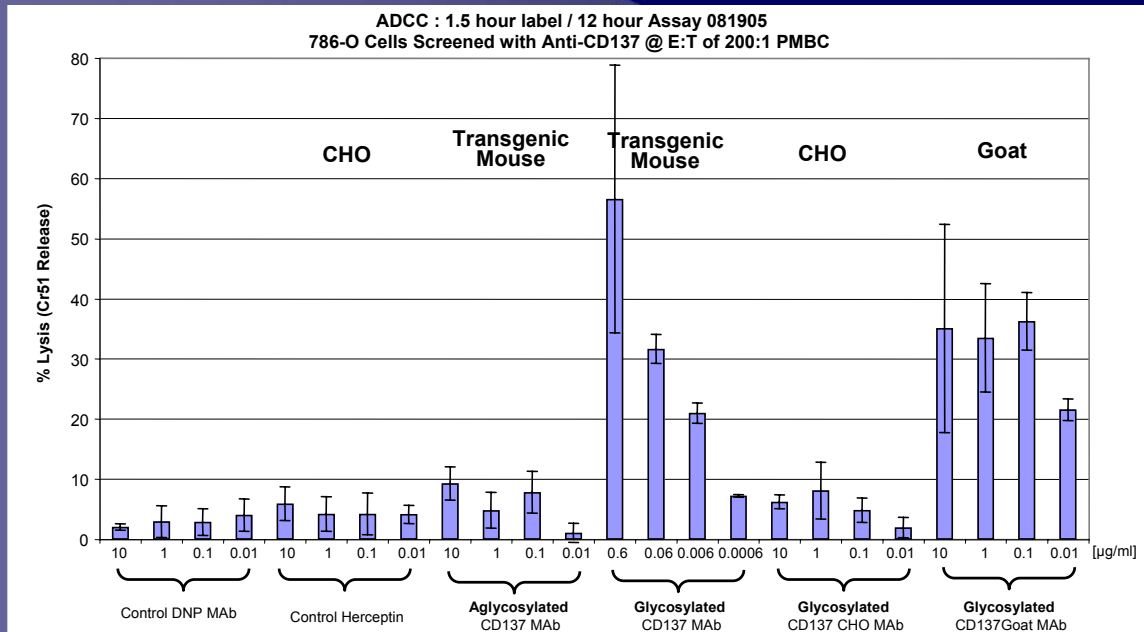
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Milk derived CD137 MAb Fc binds to CD16 more tightly than cell-culture derived MAb Fc



Transgenic Production offers enhanced ADCC



GTC Transgenic Production Technology

- Production technology → validated regulatory route (EU approved)
 - competitive COGS (low-cost producer)
 - significantly reduced capital expenditures
 - easy to scale-up (phased capital expense)
 - efficient with difficult-to-express proteins
 - enhanced ADCC due to low fucosylation
 - cost-efficient production of hi-volume proteins
 - mobile (IP favorable locations +
low-labor cost areas)
- >100 therapeutic proteins expressed in mice, goats, pigs & cows
 - > 20 monoclonal antibodies
- Outside of Cell culture and other key IP

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Merci Beaucoup!

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