Abstract No. 2730

TG20, A TRANSGENICALLY-DERIVED ANTI-CD20 MONOCLONAL ANTIBODY, EXHIBITS ENHANCED CYTOTOXICITY AGAINST CELLS WITH LOW LEVELS OF CD20

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INTRODUCTION

CD20 is a cell-surface glycoprotein that is highly expressed on most B-cells, tightly restricted to the B-cell lineage, and not expressed on either precursor lymphoid cells or the majority of plasma cells. These characteristics make CD20 an appealing target for mAb therapy of B-cell malignancies and B-cell dependent autoimmune conditions, as antibody production is maintained during therapy and B-cell regeneration post-CD20 mAb treatment is facilitated. Rituximab is the prototype; a chimeric human–mouse type-1 antibody that has proven efficacy in a wide variety of mature B-cell malignancies. However, patients do not always respond to this therapy, and it has been reported that close to 60% of follicular lymphoma patients previously treated with chemotherapy, while initially responsive, become resistant upon repeat treatment with rituximab monotherapy. Furthermore, the high cost of treatment with rituximab and other anti-CD20 mAbs severely curtails their availability to patients in emerging economies, as well as their use post treatment for maintenance therapy.

Transgenic production offers an easily scalable system for the cost-effective manufacturing of large amounts of complex therapeutic proteins. The regulatory approvals of ATryn® (recombinant antithrombin), first by the EMA (August 2006) and by the FDA (February 2009) have provided a strong regulatory validation of this production platform. Using regulatory sequences from the caprine beta-casein gene to target expression to the milk of transgenic goats, we have produced TG20, a novel chimeric anti-CD20 IgG1 exhibiting enhanced cytotoxicity against target cells.

MATERIALS AND METHODS

Transgenic Goats - Goats were maintained at a USDA registered, FDA and EMA inspected facility. The cDNA fragments encoding the heavy and light chains of TG20 were inserted into a mammary specific expression vector to obtain 2 transgenes which were co transfected into female goat fetal cells (LipofectAMINE, Gibco). Cell lines were analyzed using PCR, southern blot and FISH to confirm the presence of both heavy and light Ig chain transgenes. Nuclear transfers were performed as described previously (Melican et al. 2005; Theriogenology 63:1549). Transgene analysis of offspring (FISH, PCR and Southern blots) was conducted using genomic DNA isolated from blood and tissue samples.

CD16 assay - Jurkat CD16a cells, WIL2-S cells and PMA (Phorbol-Myristate Acetate) used respectively as effector cells, target cells and non specific activator were incubated with a dose range of TG20 antibody or Rituximab. After incubation, the Jurkat cell activation lead to IL-2 cytokine release which was quantified by ELISA. The amount of IL-2 in the supernatant cell culture is directly correlated to the ability of WIL2-S/TG20 or WIL2-S/Rituximab immune complexes to bind and activate CD16a.

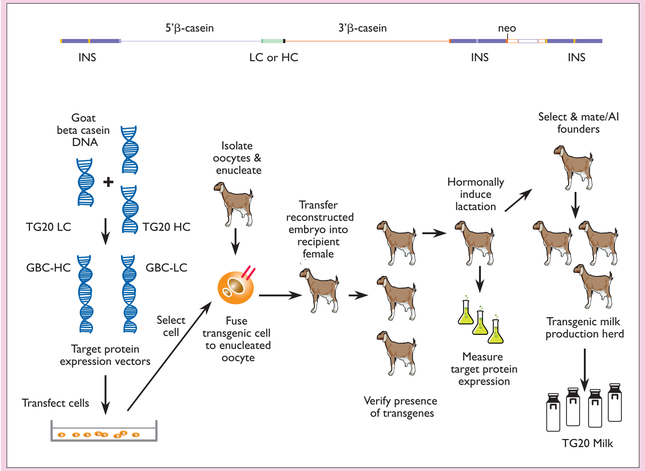
Complement-dependent cytotoxicity assay (CDC) - TG20 or Rituximab were mixed, at fixed concentrations, with WIL2-S cells expressing the CD20 antigen in the presence of human serum. In each test, 8 samples were prepared independently. After incubation at 37°C, the cell viability was estimated using a fluorescence assay (O'Brien et al. 2000; Eur J Biochem 267:5421)

Antibody-dependent cell cytotoxicity assay (ADCC) - ADCC assays were performed with calcein acetoxymethylester (AM) (Invitrogen)-labeled Raji cells and purified NK cells from healthy donors . Target cells were mixed with NK cells at an effector-target ratio of 10/1 in presence of TG20 or rituximab. Cells were incubated 4 hours at 37°C. The release of calcein AM in supernatants was measured using a microplate spectrofluorimeter (Tecan, Mannedorf-Zurich, Switzerland). Human B lymphocytes were enriched from PBMC from healthy volunteers using kits from Miltenyi.

Cynomolgus Study - Cynomolgus monkeys (*Macaca fascicularis*) ranged in weight from 3.1 to 4.4 kg. Before dosing initiation, all animals were weighed and assigned to treatment groups using a computerized randomization procedure. Dosing formulations were administered IV. Blood samples were collected twice before initiation of dosing (including spare animals) with the second sampling collected between 1 and 4 days before dosing, then on Day 1 (4 hours post-dose), and on Days 2 to 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 92. Animals were euthanized on Day 92. The blood samples were analyzed using a qualified analytical method. The lymphocytes populations were quantified (by flow cytometry, using specific antibodies against cell surface markers. Lymph nodes (LN) were collected by excisional biopsy, the lymphocytes populations were quantified as relative percentage of CD45+ lymphocytes by flow cytometry, using specific antibodies against cell surface markers. Pharmacokinetic parameters were estimated using WinNonlin pharmacokinetic, software (Pharsight Corp., Mountain View, California). A non-compartmental approach was used for parameter estimation.

RESULTS

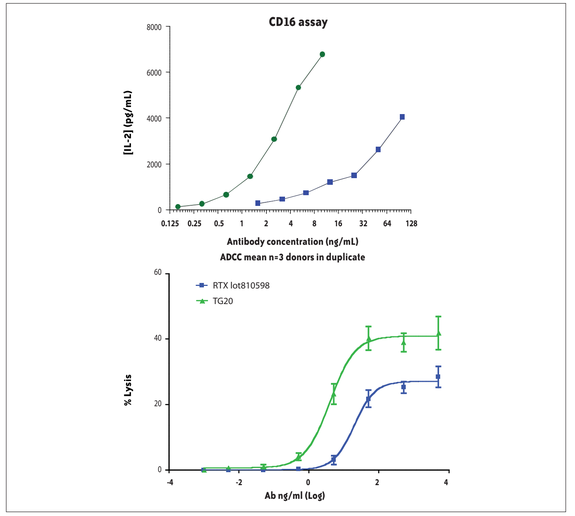
Generation of TG20 Expressing Goats



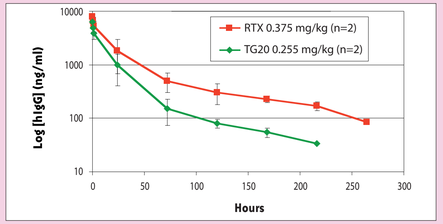
Fouder	Transgenes Goat	FISH Copy#	Induced Lactation Expression Level	Natural Lactation Expression Level
L0694	High	C25 (?)	N/A	N/A
L0716	4-5 copies	C14	> 10 g/l	10 g/l
L0737	1-2 copies	X	> 2 g/l	2.5 g/l
L0762	4-5 copies	C19	N/A	≈ 4 g/l
L0778	1-2 copies	C26	5 g/l	4-5 g/l
L0824, L0825	**3-4 copies**	**C14q1**	**2 g/l**	**2 g/l**
L0842	5 copies	C3	N/A	11-15 g/l

Functional Analysis of TG20

	CD16 activity	CD16 binding	CDC	ADCC
Tg20	100%	100%	100%	100%
Rituxan	< 2%	Not detectable	59%	6%

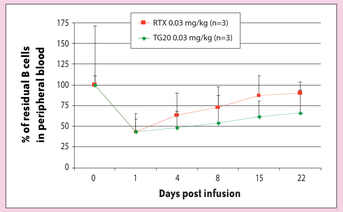


Pharmacokinetic Profile in Cynomolgus monkeys

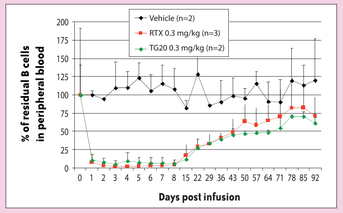


PK Parameters	Unit	RTX 0.375 mg/kg		TG20 0.255 mg/kg	
		#301	#304	#701	#704
t1/2 elim	hr	71	76	58	95
Cmax	ng/ml	8462	7387	6841	5274
AUC (0-168h)/Dose	hr*kg*ng/mL/mg	601106	331930	478180	278517
AUC (0-inf)/Dose	hr*kg*ng/mL/mg	672871	396415	500679	303662
Cl	ml/h/kg	1.49	2.52	1.99	3.29

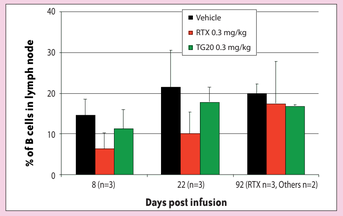
Pharmacological Activity in Cynomolgus monkeys



Blood Low-Dose



Blood High-Dose



Lymph Nodes High-Dose

CONCLUSION

TG20 is a highly active anti-CD20 mAb showing interesting characteristics in terms of cytotoxicity that make it a promising agent for indications in which rituximab is poorly active. Furthermore, the use of the cost-efficient transgenic production platform for the large-scale production of this antibody drug candidate may allow expanded access of anti-CD20 therapy to those who currently cannot afford these expensive treatments, especially in emerging market countries.

Conflict of interest Statement: Yann Echelard, Daniel P. Pollock, LiHow Chen, Nicholas C. Masiello, Jennifer L. Williams, William G. Gavin, and Harry M. Meade are employees of GTC Biotherapeutics; Catherine de Coupade, Aurélie Groseil Olivier, Frédérique Brune, Sami Chtourou are employees of LFB BIOTECHNOLOGIES. No other conflicts of interest