Abstract # 288

TG3003, an immunomodulatory anti-CD115 mAb targeting M2-macrophage polarization in the tumor microenvironment

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ABSTRACT

Cancer progression has been associated with the presence of tumor-associated M2-type macrophages (M2-TAMs) able to inhibit anti-tumor immune responses, stimulate neo-angiogenesis and facilitate metastasis. Colony-stimulating factor-1 (CSF-1, M-CSF) is a cytokine required for the survival and differentiation of myeloid cell lineages, and CSF-1 signaling is known to polarize macrophages towards the M2-type. M2-TAMs can represent the most abundant immunosuppressive cell population in the tumor microenvironment, notably recruited by CSF-1 and MCP-1/CCL2.

Transgene has developed a monoclonal antibody (mAb), TG3003, directed against the CSF-1 cell-surface receptor, CD115 (CSF-1R, M-CSFR). This mAb does not block the binding of CSF-1 to its receptor, but down-modulates CD115 signaling. In contrast to other anti-CD115 mAbs currently in development, whose modes of action rely on the blockade of ligand binding, TG3003 is not cytotoxic to normal myeloid cells that require CD115-mediated signaling for their survival. *In vitro*, TG3003 skews monocyte differentiation from M2-type macrophages towards dendritic cells, most potent antigen-presenting cells capable of stimulating efficacious T cell responses. It inhibits the secretion of MCP-1/CCL2 by differentiating macrophages and decreases their IL-6 production. Through this inhibition of M2-TAMs, TG3003 may potentiate immune responses in patients and impact on tumor progression. Moreover, due to its unique non-competitive mode of action, TG3003 does not block the physiological pathway for CSF-1 clearing from the circulation, thus avoiding the issue of toxic or rebound effects in treated patients.

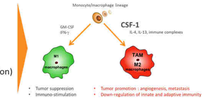
To investigate the properties of TG3003 *in vivo*, we have generated a transgenic mouse strain where the mAb epitope has been inserted into murine CD115 without affecting murine CSF-1 binding nor signaling. We present here the results of preclinical proof-of-concept experiments validating the mechanism of action and the immunomodulatory properties of mAb TG3003.

INTRODUCTION

CD115 / CSF-1 PATHWAY : A REGULATOR OF TUMOR-ASSOCIATED MACROPHAGE POLARIZATION

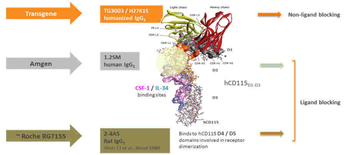
CD115 features	Roles of the CD115 /CSF-1 pathway
Also called: Colony Stimulating Factor 1 Receptor (CSF-1R), c-fms, Macrophage colony-stimulating factor receptor (M-CSFR) **Ligands:** CSF-1, IL-34 **Tyrosine kinase receptor type III** Transmembrane receptor of MW 150 kDa - 5 Ig-like extracellular domains, heavily glycosylated - Intracellular kinase domain **Ligand binding induces** dimerization and phosphorylation of CD115, followed by internalization and degradation of the complex: ***sole mechanism for CSF-1 clearing from the circulation***	Survival and differentiation of myeloid lineage cells : - monocytes, macrophages, osteoclasts, dendritic cells Involved in the **differentiation of Tumor-Associated Macrophages** (TAMs) and in their **polarization towards the M2-type**

- **Macrophages** constitute a **dominant fraction** of the immune cell population that **infiltrates** developing tumors
- **TAMs express CD115** and **their differentiation is CD115-dependent**
- Depending on specific cytokine stimulation, they can be polarized towards either M1- (tumor suppression) or M2- (tumor promotion) types



- **Circulating CSF-1 levels** are increased in many types of **solid tumors**
- **In most tumors, macrophages are considered polarized towards the M2 type**

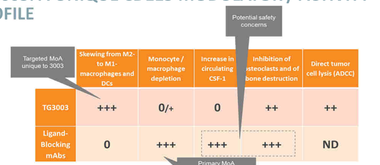
TG3003 DIFFERENTIATES FROM COMPETITORS AS THE SOLE NON-LIGAND BLOCKING MAB CURRENTLY IN DEVELOPMENT



TG3003: epitope comparison with other anti-CD115 mAbs

Scheme from Grellier B. et al, MABS 2014

TG3003: A UNIQUE CD115 MODULATOR / ACTIVITY PROFILE



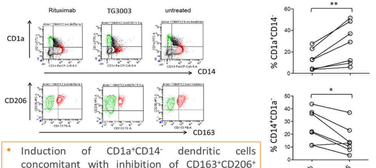
The immunomodulatory activity and an increased safety profile differentiate TG3003 from CD115 ligand-blocking mAb competitors

TG3003 INHIBITS THE DIFFERENTIATION OF HUMAN M2-MACROPHAGES *IN VITRO*

Macrophage differentiation model :

Human CD14⁺ monocytes — GM-CSF + CSF-1, 6 days →
- *Phenotype analysis*
- *Cytokine / chemokine titration*
- *Viability*

TG3003 SKEWS MONOCYTE DIFFERENTIATION TOWARDS DENDRITIC CELLS INSTEAD OF M2-POLARIZED MACROPHAGES



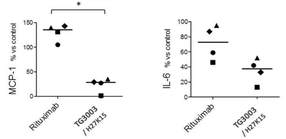
- Induction of CD1a⁺CD14⁻ dendritic cells concomitant with inhibition of CD163⁺CD206⁺ M2-macrophages
- Dendritic cells are the most potent APCs, capable of stimulating efficacious T cell responses

Results from 7 different blood donors Culture with 1 µg/ml mAb

TG3003 INHIBITS MCP-1 / CCL2 AND DECREASES IL-6 PRODUCTION BY DIFFERENTIATING MONOCYTES

❑ **MCP-1 / CCL2** implicated in TAM recruitment and tumor angiogenesis

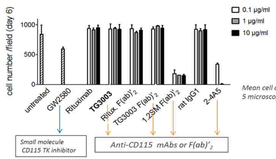
❑ **IL-6** immunosuppressive, tumor-induced cytokine



Results from 4 different blood donors Culture with 1 µg/ml mAb

- Targeting CD115 with TG3003 has drastic inhibitory effects on MCP-1 / CCL2 and IL-6, both implicated in M2-macrophage polarization and targets for cancer immunotherapy

TG3003 DOES NOT AFFECT MONOCYTE/ MACROPHAGE VIABILITY



Mean cell counts from 3 tissue culture wells 5 microscope fields counted / well

- TG3003 does not affect cell viability during monocyte differentiation
- Ligand-blocking mAbs 1.2SM or 2-4A5 are cytotoxic to differentiating monocyte-macrophages

Haegel H. et al, MABS 2013

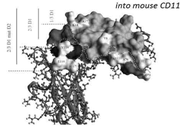
GENERATION OF A CD115-KI MOUSE MODEL

TG3003 EPITOPE INSERTED INTO MURINE CD115

TG3003 reacts only with human or chimpanzee CD115

→ a CD115-humanized (knock-in) transgenic mouse was generated for *in vivo* PoC and tox studies

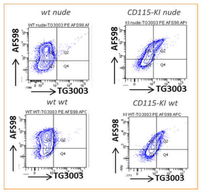
TG3003 epitope inserted into mouse CD115



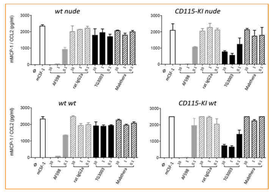
Transgenic mice express a selected human / murine chimeric CD115 form, which
- ➢ *binds mCSF-1 with same affinity as mCD115*
- ➢ *binds TG3003 with same affinity as hCD115*
- ➢ *transduces CSF-1 signaling in vitro*

CD115-KI mice were generated on both immuno-competent (C57BL/6) and -deficient (nude) backgrounds

TG3003 BINDS BONE-MARROW DERIVED MACROPHAGES FROM CD115-KI MICE AND INHIBITS THEIR PRODUCTION OF MCP-1 / CCL2



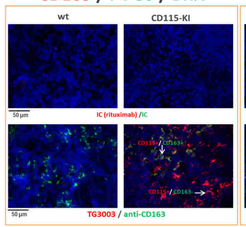
Bone-marrow derived macrophages differentiated with mGM-CSF were analyzed by flow cytometry with TG3003 and AF598 (anti-mouse CD115) (gated on live cells).



Murine MCP-1 / CCL2 was titrated in culture supernatants from bone-marrow derived macrophages differentiated with mCSF-1 in the presence of anti-CD115 or control mAbs (triplicates)

TG3003 BINDS TISSUE MACROPHAGES FROM CD115-KI MICE

CD163 / F4-80 / DNA — **CD115 / CD163 / DNA**

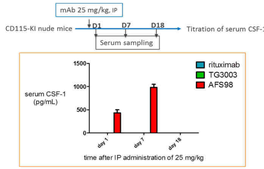


Immunohistochemical analysis of frozen spleen sections from CD115-KI vs wt nude mice

wt: wild type WP : White Pulp RP : Red Pulp IC : Isotype Control

TG3003 : *IN VIVO* PROOF-OF-CONCEPT IN CD115-KI HUMANIZED MICE

TREATMENT WITH TG3003 DOES NOT INCREASE SERUM CSF-1



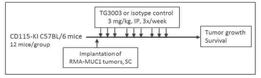
In contrast to ligand-blocking anti-CD115 mAbs (like AFS98),
TG3003 does not block the physiological pathway for CSF-1 internalization and degradation
-> no increase in circulating CSF-1 -> no risk of rebound effect

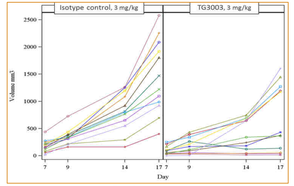
TG3003 AS IMMUNOMODULATOR : THERAPEUTIC EFFECT IN THE RMA-MUC1 TUMOR MODEL

Inhibition of M2-macrophages and skewing towards M1-macrophages and DCs may stimulate anti-tumor immune response

RMA lymphoma cell line :
- MHC class I⁺
- Immunogenic, aggressive tumor growing in C57BL/6 mice
- Tumor rejection is CD4⁺- and CD8⁺-T-cell mediated

-> effect of TG3003 tested in CD115-KI C57BL/6 mice implanted with RMA-MUC1 tumors :

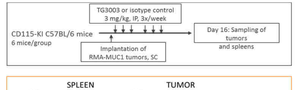


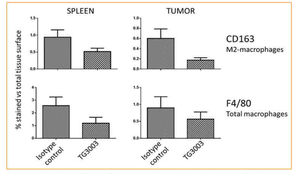


Effect of TG3003 on tumor growth in individual mice
Statistically significant difference between groups
N=1 experiment

Control of tumor growth in mice treated with TG3003 -> *suggests improvement of anti-tumor immune response*

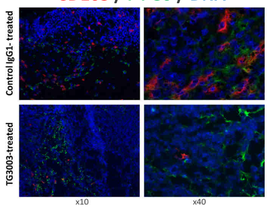
TG3003 INHIBITS M2-MACROPHAGES IN TUMOR-BEARING MICE





CD115-KI C57BL/6 mice bearing RMA-MUC1 tumors were treated with TG3003 or isotype control (3 mg/kg).
Tumors and spleens were sampled after 16 days. Frozen tissue sections were immunostained for markers CD163 and F4/80.
N=1 experiment

CD163 / F4-80 / DNA



Immunohistochemical analysis of RMA-MUC1 tumor sections from CD115-KI mice treated with TG3003 or isotype control (3 mg/kg)

Treatment with TG3003
- **decreases CD163⁺ (M2-polarized) TAMs**
- **without depleting CD163⁻ (M1-polarized) F4/80⁺ TAMs**

-> preservation of immunostimulatory antigen-presenting cells