TGD001, a VWF-targeted thrombolytic, is safe and effective in animal models for the treatment of thrombotic thrombocytopenic purpura

C Tersteeg[1], S Mc Fie[2], D Versluis[2], A Delmote[1], K Vercruysse[2], K Wosikowski[2], G van 't Klooster[2], K Vanhoorelbeke[1], S de Maat[2]
[1] KU Leuven Campus Kulak, Kortrijk, Belgium
[2] Targed Biopharmaceuticals B.V., Utrecht, The Netherlands

INTRODUCTION

Thrombi are heterogeneous in composition, which limits the efficacy of available thrombolytic treatments. Hereto, Microlyse was created (De Maat et al., 2022), containing an anti-von Willebrand factor (VWF) VHH antibody coupled to the protease domain of urokinase. This design allows Microlyse to bind to VWF-containing thrombi and locally activate plasminogen, thereby inducing thrombolysis. Microlyse was effective in several thrombotic models with various thrombi compositions. For its clinical application, Microlyse was updated to TGD001; containing a humanized VHH and a modified urokinase protease domain (Figure 1). TGD001 is being developed for various thrombotic indications, including thrombotic thrombocytopenic purpura (TTP), a VWF-driven thrombotic microangiopathy caused by ADAMTS13 deficiency.

We here demonstrate that:

- TGD001 has a low potential for driving anti-therapeutic immune response in humans.
- TGD001 is well-tolerated in rats, even with repeated dosing up to 12 mg/kg.
- TGD001 demonstrates a half-life of 2 hours in rats.
- TGD001 rapidly reduces thrombocytopenia and tissue damage in murine TTP models.

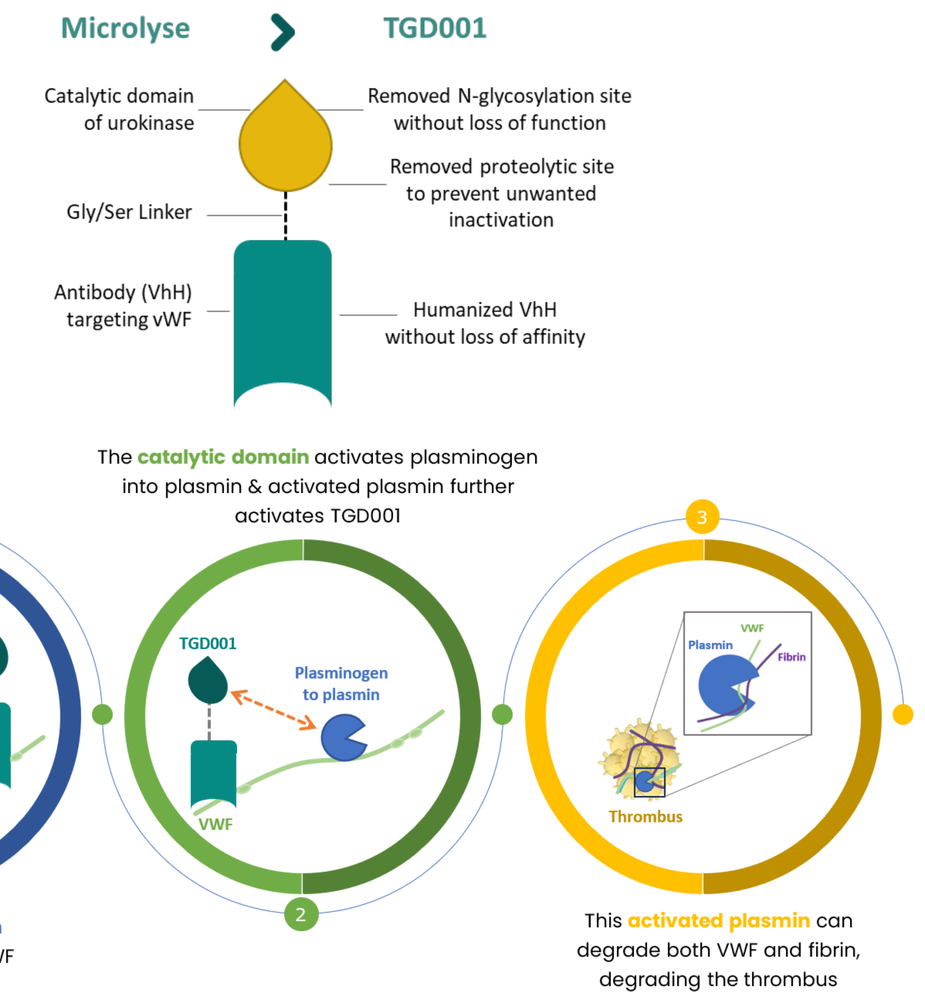

Figure 1: Evolution of TGD001 and its mechanism of action

RESULTS

IMMUNOLOGICAL POTENTIAL OF TGD001

Using in silico prediction (EpiMatrix and JanusMatrix tools, as described by de Groot et al., 2023), TGD001 demonstrates lower immunological potential compared to Microlyse (Figure 2). This is due to the humanization of the VHH sequence as modifications in the urokinase catalytic domain do not affect immunological potential.

Overall, TGD001 falls in the low range of the EpiMatrix Protein scale, indicating a minimal potential for driving anti-therapeutic immune response in humans.

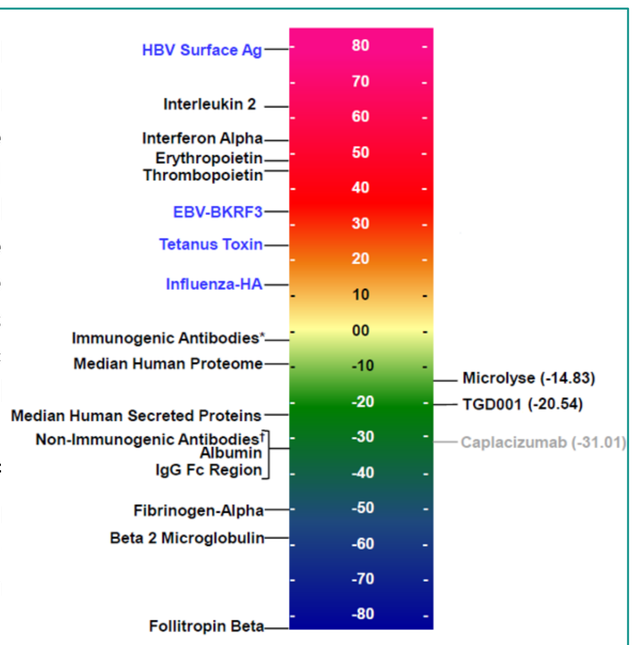

Figure 2: EpiMatrix Protein Scale: TGD001 and Microlyse

TGD001 TOXICOLOGY AND PHARMACOKINETICS IN RATS

The compatibility of TGD001 with different species was analyzed for the preclinical species selection. TGD001 binds rat VWF with an affinity (KD) of 24 nM. TGD001's plasminogen activation potential in plasma is only 3-fold lower in rats compared to humans, making rats an appropriate rodent safety model for TGD001. In the dose-range finding study, TGD001 was administered to Wistar Han IGS rats at 1, 4, or 12 mg/kg on Days 1, 3, 5, and 8 by intravenous bolus injection to examine toxicology. No TGD001-related changes in clinical phenotype, body weight, or clinical pathology were observed, indicating that TGD001 is well tolerated (Table 1). Importantly, the coagulation markers, including PT, APTT, and fibrinogen levels, were unaffected by TGD001. Regarding pharmacokinetics, TGD001 was quantifiable in rats up to 8 hours post-dose with a plasma half-life of 2 hours.

Table 1: The effects of repeated administrations of TGD001 on toxicology measures.

Measure	Test-item related effects
Mortality	No unscheduled deaths
Clinical observations	No effects
Body weight	No effects
Hematology	No effects
Coagulation	No effects
Clinical chemistry	No effects
Macroscopic pathology	No effects
Organ weight	No effects
Microscopic evaluations	Minimal to mild findings observed in the liver, related to the physiological metabolic adaption to the test-item

TGD001 ATTENUATES THROMBOCYTOPENIA AND TISSUE DAMAGE IN TTP MODELS

The efficacy of TGD001 was investigated in an *Adamts13*$^{-/-}$ knockout mouse model of congenital TTP (cTTP) and an antibody-mediated mouse model of immune TTP (iTTP; Figures 3 & 4, respectively). In both models, TTP symptoms were triggered by the administration of recombinant human VWF at T-0. Fifteen minutes hereafter, mice received vehicle or TGD001 (as indicated by the dotted line).

In both models, mice treated with vehicle present with the expected TTP-associated characteristics including thrombocytopenia and elevated tissue damage, measured via lactate dehydrogenase (LDH) levels. Treatment with TGD001 attenuates thrombocytopenia and normalizes tissue damage within 24 hours in both models. Analysis of additional time points in the cTTP model shows increased platelet counts and lowered LDH levels within one hour of TGD001 administration (Figure 3), indicating the rapid initiation of thrombolysis and reduction of tissue damage.

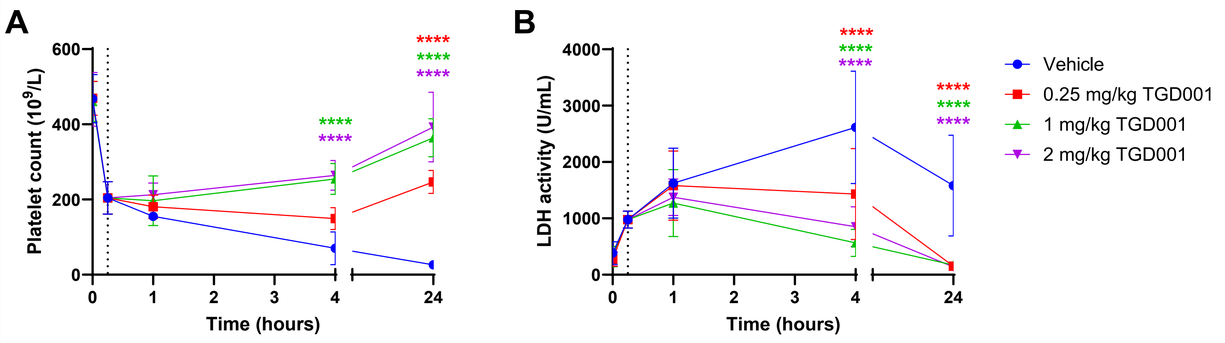

Figure 3: TGD001 rapidly attenuates A) thrombocytopenia and B) tissue damage in a murine cTTP model with *Adamts13*$^{-/-}$ mice.

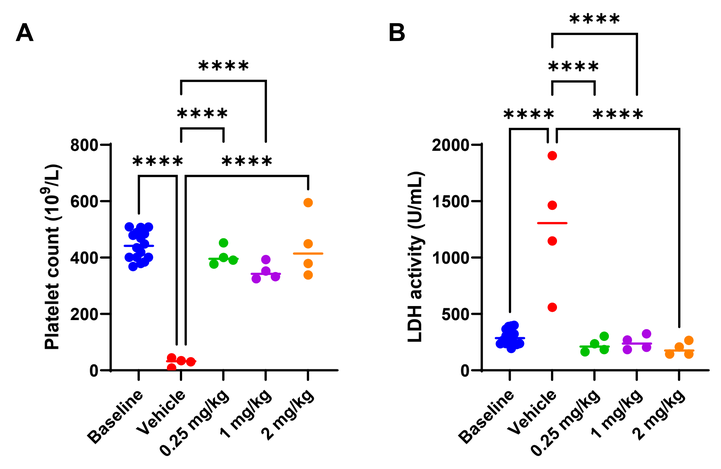

Figure 4: TGD001 rapidly attenuates A) thrombocytopenia and B) tissue damage at 24 hours post-TTP attack in an antibody-mediated murine iTTP model.

CONCLUSION

TGD001 rapidly attenuates thrombocytopenia and tissue damage in cTTP and iTTP models. Moreover, TGD001 shows a low immunological potential in humans and causes no treatment-related adverse effects in rats.

CONTACT

Email: Info@Targedbio.com
Website: www.targedbiopharmaceuticals.com

References:
de Maat, Steven, et al. "Microlyse: a thrombolytic agent that targets VWF for clearance of microvascular thrombosis." *Blood, The Journal of the American Society of Hematology* 139.4 (2022): 597-607.
de Groot, Anne S., et al. "Does human homology reduce the potential immunogenicity of non-antibody scaffolds?." *Frontiers in Immunology* 14 (2023): 1215939.