#6310 Phylogenetic conservation of primate Trop-2 underscores favourable pharmacokinetics and lack of toxicity of the cancer-selective Hu2G10 anti-Trop-2

Saverio Alberti, MD, PhD[1,2*]; Martina Ceci[3,4]; Ludovica Pantalone[3]; Marco Trerotola, PhD[3,4]; Emanuela Guerra, PhD[2,3,4]

[1]Unit of Medical Genetics, Department of Biomedical Sciences - BIOMORF, University of Messina, Messina, Italy [2]Mediterranea Theranostic, Lanciano, Italy [3]Laboratory of Cancer Pathology, CAST, "G. d'Annunzio University" of Chieti-Pescara, Chieti, Italy [4]Department of Medical, Oral and Biotechnological Sciences, "G. d'Annunzio University" of Chieti-Pescara, Chieti, Italy *alberti.saverio@gmail.com

www.mediterranea-theranostic.com/en/

ABSTRACT

Trop-2 is a transmembrane signal transducer that activates growth-signaling networks that converge on Akt, ERK, Cyclin D1, NFkB (1). We discovered that Trop-2 cleavage by ADAM10 is an activator switch of cancer growth and metastatic diffusion (2) and exposes novel target epitopes that are inaccessible in the unprocessed wtTrop-2. The Hu2G10 mAb selectively binds these epitopes with an affinity that is ≈10,000-fold higher than that for the unprocessed molecule (3). To validate a reliable model for Hu2G10 binding *in vivo,* we explored Trop-2 phylogenetic conservation in primates. *Pan troglodites* (chimpanzee), *Papio anubis* (baboon), *Macaca mulatta/fascicularis* (rhesus/cynomolgous monkey), *Callithrix jaccus* (marmoset) *TROP2/TACSTD2* gene and protein sequences were compared. The only difference between *Pan troglodytes* and human Trop-2 is one additional leucine in the *Pan* leader peptide, making the mature Trop-2 identical to that in man. The *Macaca mulatta/fascicularis* Trop-2 sequences are 97% identical, 100% similar to the human sequence. Three-dimensional modeling revealed polymorphic residues usage versus activation-driven structural rearrangement of Trop-2. COS-7 and HEK-293 cells transfected with individual primate Trop-2 showed efficient recognition by Hu2G10 in all tested species. Immunohistochemistry analysis of normal Macaca tissues showed Trop-2 expression patterns essentially corresponding to the human ones. Intravenously-injected Hu2G10 at doses of 5 to 10 mg/kg was well tolerated by cynomolgous monkeys. No neurological, respiratory, digestive and urinary adverse effects were observed, and no body-weight loss occurred during the 28-day observation. No alterations of blood monocyte, neutrophil and basophil counts nor significant changes in biochemical parameters were detected. A pharmacokinetic (PK) study was carried out. Serum concentration of Hu2G10 was determined using ELISA assays with an anti-idiotypic antibody. At the higher dosing of 10 mg/kg Hu2G10 serum values reached a peak 2 hours after the injection (13281±4686 ng/ml). The concentration of Hu2G10 then diminished gradually, and reached baseline levels on day 21. The serum concentration peak of 5 mg/kg Hu2G10 was 5759±1729 ng/ml, and reached baseline levels on day 14. Thus, Hu2G10 is stable in plasma, and is detectable in the circulation up to three weeks after the infusion ($t_{1/2}$=6.5 days). Taken together, these findings validate primate models as reliable for assessing Hu2G10 *in vivo* toxicity and PK. The lack of toxicity and favourable PK parameters candidate the cancer specific Hu2G10 as first-in-class anti-Trop-2 mAb.

INTRODUCTION

Trop-2-targeting anticancer therapy is heavily hampered by Trop-2 expression in normal tissues. Recently we showed that Trop-2 undergoes cancer-specific functional activation via R87-T88 proteolytic cleavage (1,2). To exploit this cancer vulnerability we generated the first-in-class 2G10 monoclonal antibody (mAb). 2G10 was humanized by state-of-the-art CDR grafting, leading to the Hu2G10 anti-Trop-2 mAb. Hu2G10 specifically targets the cleaved Trop-2, (Kd <10-12 M versus a Kd 3.16x10-8 M for the uncleaved Trop-2 in normal tissues), elicits an efficient ADCC response and shows potent anticancer activity in vivo (3), with no toxicity in murine models (3). We the went on to investigate toxicity and pharmacokinetics of the Hu2G10 mAb in a validated model of non-human primate.

References

1. Guerra E. et al. Oncogene 41, 1795-1808 (2022). 2. Trerotola M. et al. Neoplasia 23, 415-428 (2021). 3. Guerra E. et al. Mol. Cancer Ther. 2023 Mar 15:MCT-22-0352.

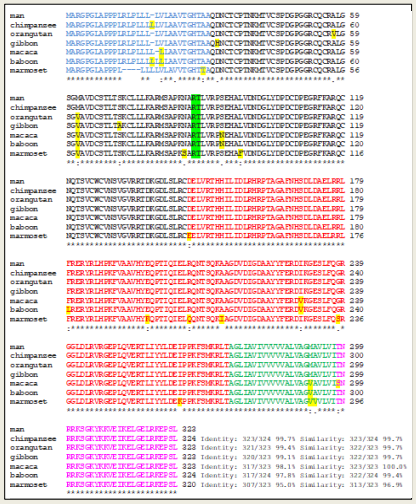


Figure 1. Phylogenetic analysis of Trop-2 conservation in primates. RefSeq Trop-2 sequences were retrieved from the NCBI database and aligned using Clustal O. Human Trop-2 is shown at the top. Blue: leader peptide; black: globular domain; red: stem region; green: transmembrane domain; magenta: cytoplasmic tail Yellow highlight: mismatches. Green highlight: conserved R87-T88 cleavage site. % identity and similarity with respect to the human sequence is listed at the bottom. *Macaca mulatta* and *fascicularis* (rhesus/cynomolgous monkey), share the same Trop-2 sequence, which is 97% identical, 100% similar to the human one.

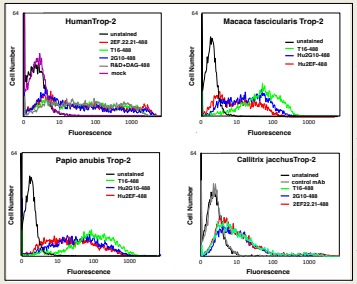


Figure 2. Efficient recognition of monkey Trop-2 by the 2G10 mAb. Flow-cytometry analysis of monkey fibroblast COS-7 cells transfected with expression vectors for human (as positive control), or monkey (cynomolgous, baboon, marmoset) Trop-2. 2G10 staining is indicated by the red profile. Framework (T16) and 2EF mAbs and R&D anti-Trop-2 goat polyclonal Ab were used as benchmarks. Mabs were directly conjugated to Alexa-488. R&D anti-Trop-2 binding was revealed by donkey-anti-goat-488 secondary Ab.

RESULTS

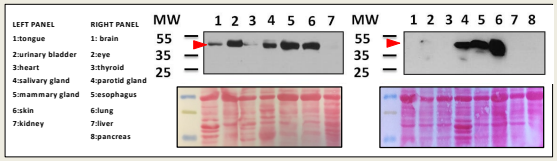


Figure 3. Lack of Trop-2 cleavage in normal monkey tissues. Western blot analysis of Trop-2 expression in normal rhesus monkey tissues (top panels). Ponceau red staining as control of protein loading is shown (bottom panels). MW markers are indicated. Accumulation of uncleaved Trop-2 band in most of the analyzed tissues is detected (red arrowhead), and absence of Trop-2 expression in kidney, brain, eye, liver and pancreas.

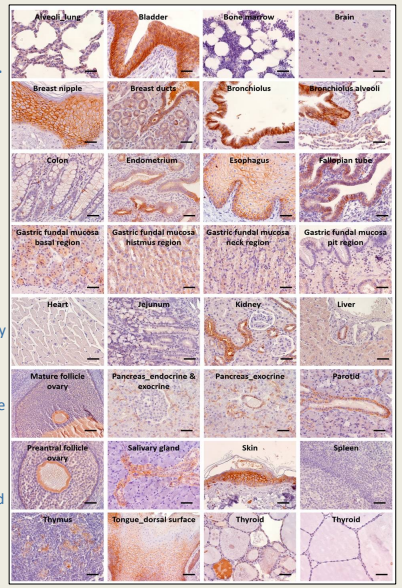


Figure 4. Trop-2 expression patterns in normal monkey tissues. IHC analysis of Trop-2 expression was performed on formalin-fixed, paraffin-embedded rhesus monkey normal tissues. Brown deposits indicates the presence of Trop-2. Scale bars, 50 µm. Highest Trop-2 expression was found in differentiated layers of multistratified epithelia (skin, bladder, mammary glands, tongue, bronchiolus alveoli and esophagus). Intermediate levels were found in pancreas, salivary glands, parotid, followed by liver (bile ducts). No Trop-2 expression was detected in bone marrow, intestine, brain, spleen and striated and cardiac muscle.

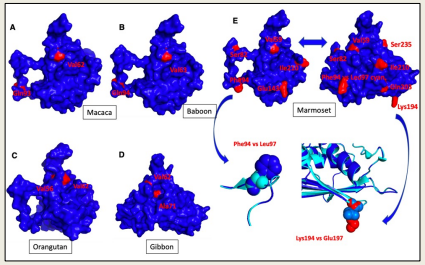
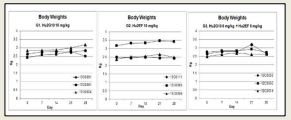


Figure 5. 3D modeling of primate Trop-2. Target sequence and a template structure file in PDB format were aligned. Models were built based on the target-template alignment using ProMod3. Insertions and deletions were remodelled using a fragment library. Side chains were then rebuilt. The geometry of the resulting model was regularized by using a force field. The global and per-residue model quality was assessed using the QMEAN scoring function.



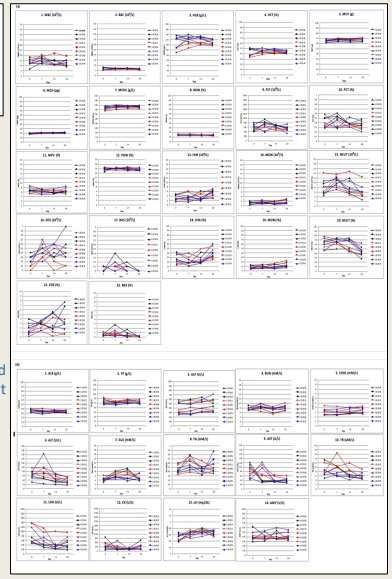


Figure 5. Lack of toxicity of Hu2G10, Hu2EF. Cynomolgous monkeys received IV: Hu2G10 at 10 mg/kg, Hu2EF at 10 mg/kg and a combination of the two mAbs 5 mg/kg each. (top) Body weight measurements. No body-weight loss was observed during the entire observation. (right) Hematological and biochemical parameters in peripheral blood samples. Values for individual monkeys are shown. Hu2G10 group: red; Hu2EF: black; combination: blue. No significant alterations of white and red blood cell counts (WBC and RBC), hemoglobin (HGB), hematocrit (HCT), platelets (PLT) and monocytes, neutrophil and basophil counts were observed. No significant changes of albumin, total protein (TP), alkaline phosphatase (ALP), blood urea nitrogen (BUN), cholesterol, alanine aminotransferase (ALT), glucose, triglyceride (TG), aspartate aminotransferase (AST), total bilirubin (TB), lactate dehydrogenase (LDH), creatinine kinase (CK), serum creatinine (sCr) and amylase (AMY) were detected. No neurological, respiratory, digestive and urinary adverse effects were observed.

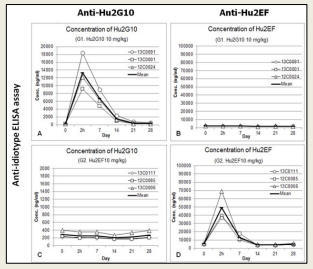


Figure 3. PK evaluation of Hu2G10 mAb in Cynomolgous monkeys. Serum samples were collected on day 0, (2-hour before and after infusion), 7, 14, 21 and 28. Serum concentrations of Hu2G10 and Hu2EF were determined using ELISA assays with proprietary antiidiotypic mAbs. Hu2G10 and Hu2EF serum concentration reached a peak 2 hours after infusion, then diminished gradually, and reached baseline levels on day 21. Peak values for Hu2G10 were 13281±4686 ng/ml (10 mg/kg dosing) and 5759±1729 ng/m (5 mg/kg dosing). Thus Hu2G10 is stable in plasma, and is detectable in the circulation up to three weeks after the infusion, with $t_{1/2}$=6.5 days.

CONCLUSIONS

- Non-human-primate (NHP) Trop-2 expression in normal tissues paralles that of human Trop-2.
- NHP Trop-2 is efficiently recognized by the cancer specific Hu2G10 mAb.
- Toxicity studies in cynomolgous monkey show no adverse effects of Hu2G10 at dosing up to 10 mg/kg
- Hu2G10 is stable in plasma, with a measured $t_{1/2}$ of 6.5 days.

The lack of toxicity and favourable PK parameters candidate the cancer specific Hu2G10 as first-in-class anti-Trop-2 mAb.