Early discovery due diligence of immuno-inflammation target through multi-pronged approaches

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Discovery on TARGET VIRTUAL | September 16-18 2020

Synergism of *In silico* expertise for comprehensive Target Analysis: a successful kick start to a discovery program

AI driven data mining and analysis

Open-source and licensed databases

Scientific curation by domain experts

Automated pipelines

The advent of high throughput omics technologies has enabled simultaneous exploration of disease-causing factors as drug targets. Our assessment aids informed decision making at the initiation of a discovery program for novel first and best-in-class drugs.

By extensive diligence involving artificial intelligence-guided data mining and informatic analysis we provide **evidence-based insight on specificity, efficacy and safety of a therapeutic drug target.**

We offer:

- literature and database-based 360 evaluation including understanding of **mechanism of disease progression,**
- homolog analysis driven **on- and off-target effect prediction,**
- **expression and localization,**
- evaluate **co-targeting need** for better efficacy
- extensive coverage of **competitive landscape**

Evidence-based prediction of adverse events in humans

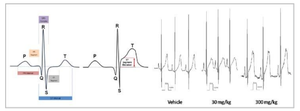
Literature-based analysis

In mice: cardiac structure impaired, function unaffected

- Cleavage of versican defective in the heart of high fat diet-treated *Adamts5*$^{-/-}$ mice.
- Knockout mice had increased in diastolic posterior wall thickness (0.94 ± 0.023 vs. 0.82 ± 0.036 mm; p = 0.0056), and
- And increased left ventricle volume (47 ± 4.5 vs. 31 ± 2.5 µL; p = 0.0043).

Hemmeryckx B et al Cell Biol Int. 2019;43(6):593-604

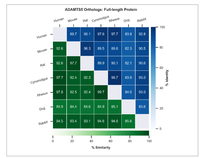
In cynomolgus monkey : cardiac function impaired



Sewell F et al MAbs. 2017;9(5):742-755.

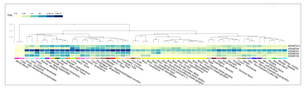
Informatics analysis

Global sequence alignment with respect to full length protein



Human ADAMTS5 shares highest sequence identity with the cynomolgus and rhesus monkey sequence (>97%), followed by mouse and rat. Adverse effects observed in cynomolgus monkey, therefore, have a possibility of being imitated in humans

Pan tissue expression analysis among ADAMTS5, ADAMTS1, ADAMTS4, ADAMTS15 and ADAMTS8 revealed differential expression pattern *Source: GTEx*



- GTEx does not assay expression levels of ADAMTS5 in cartilage tissues, but scientific literature reports constitutive level of expression of ADAMTS5 protein and mRNA in chondrocytes and articular cartilage tissue*
- ADAMTS4 has medium to low expression in visceral omentum adipose tissue, followed by lower expression in female reproductive tissues
- ADAMTS1 has a wider range of high expression seen in most tissues

** Moulharat N et al. Osteoarthritis Cartilage. 2004;12(4):296-305, Tetsunaga T et al. Osteoarthritis Cartilage. 2011;19(2):222-232, Mao G et al. Cell Physiol Biochem. 2017;44(1):38-52.*

>50% epitope sequence conservation among ADAMTS5,1, and 8 for anti-ADMATS4 mAb (GFC301 by Genfrontier)

Paralogs	Epitope sequence										Percentage conservation across paralogs
ADAMTS4	Y^{558}	C	E	G	R	R	T	R	F	R^{568}	100%
ADAMTS5	Y	C	T	G	K	R	A	I	Y	R	50%
ADAMTS15	Y	C	E	G	V	R	V	K	Y	R	40%
ADAMTS8	Y	C	L	G	R	R	A	K	Y	Q	50%
ADAMTS1	Y	C	E	G	K	R	V	R	Y	R	80%
Residue percentage conservation	100%	100%	60%	100%	40%	100%	20%	40%	20%	80%	

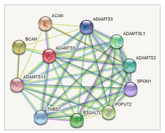
- GFC301 binds to the TSP type-1-1 domain of ADAMTS4 and reduces aggrecanase activity of both ADAMTS4 and ADAMTS5. (Patent: US10640573B2).

**ADAMTS4 epitope sequence is used as the base for calculating percentage conservations across paralogs and residue wise conservation.*

Target Safety Assessment

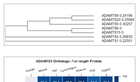
Target biology in relevance to toxicology

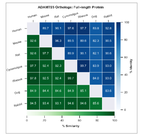
- Altered function in disease, network-analysis for novel targets
- Expression profile (canonical and alternate isoforms) - normal and disease tissues in human and animals



Target homologs in relevance to toxicology

- Targeted domain - sequence conservation across homologs
- Targeted binding domain – structural homology across protein families

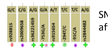




Target Safety Assessment

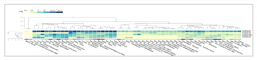
Risk Mitigation Plan

Polymorphisms/mutations in target

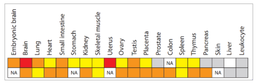


SNPs/mutations associated adverse affects in humans

Toxicities associated with expression



Safety risks assessment by organ system



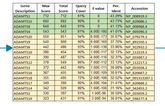
Estimating off- target effects : paralog identification

Human canonical protein sequence of human ADAMTS5 (NCBI gene) blasted against the RefSeq protein database

70 % query coverage and 30 % identity

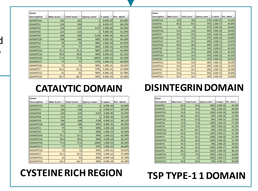
17 paralog proteins are obtained w.r.t full length protein

Blast result



Domain boundaries of catalytic, disintegrin, TSP type-1 1 domain and cysteine rich region, are extracted from UniProt for all 17 paralogs.

Individual domains are blasted for all 17 proteins in four separate blast analyses.



70 % query coverage and 40 % identity

Protein/Domain	Paralogs- arranged in decreasing order of sequence identity
Full length	ADAMTS – 1, 15, 8, 4
Catalytic domain	ADAMTS – 1, 15, 8, 4, 9, 7, 20, 6
Disintegrin domain	ADAMTS – 1, 15, 9, 18, 7, 20, 17, 19, 6, 8, 16, 4
TSP type-1 1 domain	ADAMTS – 1, 8, 15, 4, 12, 6, 9, 18, 20, 7, 10, 3, 14, 2, 16, 17, 19
Cysteine-rich region	ADAMTS – 15, 1, 4, 8, 9, 20, 3, 2, 14, 16, 18, 17
Spacer region	ADAMTS – 4, 1, 8

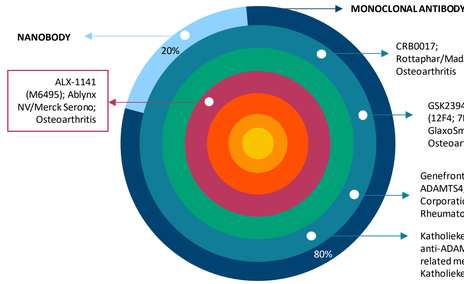
Paralog set identified w.r.t. catalytic, disintegrin, TSP type-1 1 domain and cysteine rich region, are taken forward for further analysis

70 % query coverage and 40 % identity

70 % query coverage and 30 % identity

Nanobody-mediated targeting is the preferred modality for better efficacy

Competitive landscape* of ADMATS5 large molecule inhibitors



Advantages of therapeutic nanobody for ADAMTS5

Competitive landscape reveals ADAMTS5 targeting nanobody in the most advanced phase of clinical studies.

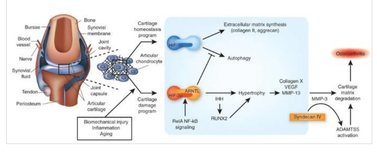
- Nanobody inhibits just the metalloproteinase **catalytic activity** without eliciting an immune response
- Fc-mediated **effector functions are minimized** (antibody and complement-mediated cytotoxicity minimized)
- Secreted and active form of ADAMTS5 is **better accessed** by the nanobody (due to smaller size)
- **Bioavailability and stability** of nanobody higher than that of monoclonal antibody

Morrison, News: Nature Review Drug Discovery, 2019

*Competitive landscape: Competitive landscape identifies direct or indirect competitors to help comprehend their market, strengths, and weaknesses.

ADAMTS5 as a target for treating inflammation indications observed in osteoarthritis

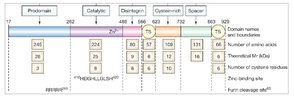
- ADMATS5 (A Disintegrin-like and Metalloproteinase with Thrombospondin-1 motifs) is a metalloproteinase that cleaves extracellular proteoglycans
- The major substrates being: aggrecan, versican, brevican, biglycan, and reelin
- The aggrecan digesting property of ADAMTS5 associates it with the development of osteoarthritis
- ADMATS family has 19 members with multiple substrate specificity which defines its range of biological functions



Husa M et al. Nat Med. 2010;16(6):641-644.

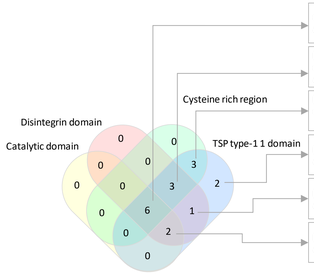
ADAMTS5 protein domain organisation

- ADAMTS5 is comprised of prodomain, catalytic, disintegrin, TSP-type 1, TSP-type 1 2 domain, cysteine rich region and the spacer region.
- Targeting catalytic, disintegrin, TSP-type 1 domain, spacer and cysteine rich region individually can lead to reduction in the catalytic activity of ADAMTS5.



Domain structure and features of ADAMTS5: Structural motifs have been shown in the diagram, with domain boundaries, theoretical molecular weight (Mr), number of amino acids, cysteine residues, zinc binding domain and furin recognition sequence (Source: Fosang AJ and Little CB. Nat Clin Pract Rheumatol. 2008;4(8):420-427)

Prediction of enhanced efficacy : co-targeting of functional paralogs



Catalytic domain ∩ Disintegrin domain ∩ Cysteine rich region ∩ TSP type- 1 1
ADAMTS1, ADAMTS15, ADAMTS20, ADAMTS4, ADAMTS8, ADAMTS9

(Disintegrin domain ∩ Cysteine rich region ∩ TSP type- 1 1 domain) - (Catalytic domain)
ADAMTS16, ADAMTS17, ADAMTS18

(Cysteine rich region ∩ TSP type- 1 1 domain)-(Catalytic domain ∪ Disintegrin domain)
ADAMTS14, ADAMTS2, ADAMTS3

(TSP type- 1 1) – (Catalytic domain ∪ Disintegrin domain ∪ Cysteine rich region)
ADAMTS12, ADAMTS10

(Disintegrin domain ∩ TSP type- 1 1) – (Catalytic domain ∪ Cysteine rich region)
ADAMTS19

(Catalytic domain ∩ Disintegrin domain ∩ TSP type- 1 1) – (Cysteine rich region)
ADAMTS6, ADAMTS7

- Common set of paralogs w.r.t. all the domains are ADAMTS1, ADAMTS4, ADAMTS15, ADAMTS8, ADAMTS20 and ADAMTS9.
- Out of these paralogs ADAMTS20 and ADAMTS9 possess lower identities compared to **ADAMTS1, ADAMTS4, ADAMTS15** and ADAMTS8.
- ADAMTS4 andADAMTS5 are the major aggrecanases implicated in arthritis *.
- With significant sequence and functional similarity, co-targeting of ADAMTS4 and ADAMTS5 may be proposed for better efficacy.

** Tortorella MD, Malfait AM. Curr Pharm Biotechnol. 2008;9(1):16-23*

Subcutaneous administration of ADAMTS5 biotherapeutic may avoid off-target effects - by avoiding ADAMTS1 binding

Marginal alleviation of adverse events based on route of administration

	Subcutaneous	Intravenous
Toxicology study of anti-ADAMTS5 antibody in cynomolgus monkey	Dose – 30 mg/kg Adverse events: • ST segment elevation • Day1 – increased intraventricular conduction delay and increased frequency of isolated premature ventricular contractions • Day 21- increased mean arterial pressure	Dose – 300 mg/kg Adverse events: • ST segment elevation • focal endocardial hemorrhage in the left ventricle • **Day1 and Day7** – increased frequency of isolated premature ventricular contractions • **Day 14 and Day 21** - increased mean arterial pressure
Literature-based advantages	• Prolonged rate of absorption • Self administered by patients • Hospital and clinical cost savings • Localized administration might avoid off-organ and off-target toxicity	• Higher bioavailability • Higher probability of injection-site reactions and pain

Sewell F et al MAbs. 2017;9(5):742-755.

Summary of results

Adverse event prediction (and mitigation):

- Mitigation plan for probable AE in human – **testing in non-human primates**

Minimizing off-target and off-organ toxicity:

- Off target effect with respect to ADAMTS1 paralogs can be minimised through **subcutaneous mode of administration**
- Targeting the catalytic domain, TSP type-1 or cysteine rich domain may lead to ADAMTS1 dependent off-organ toxicity
- Targeting **spacer domain** may lead to higher efficacy and lower off- organ toxicity

Maximizing efficacy:

- Co-targeting of paralogs – **ADAMTS4 & ADAMTS5** due to: similar aggrecanase activity, organ-wise expression pattern, sequence conservation across domains
- SC is preferred route of administration for reduction of off-organ effect.

Large molecule-based activity inhibition:

- **Nanobody-mediated targeting** is better than monoclonal antibody for secreted proteins.