

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

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



AI driven data mining and analysis

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:

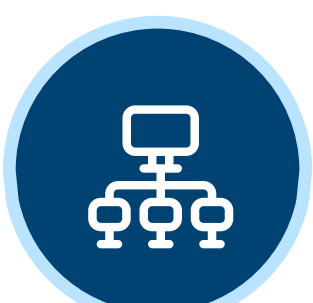
- literature and database-based 360 evaluation including understanding of **mechanism of disease progression**,
- homolog analysis driven **on- and off-target effect prediction**,
- expression and localization**,
- evaluate **co-targeting need** for better efficacy
- extensive coverage of **competitive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



Scientific curation by domain experts



Open-source and licensed databases



Automated pipelines

## Evidence-based prediction of adverse events in humans

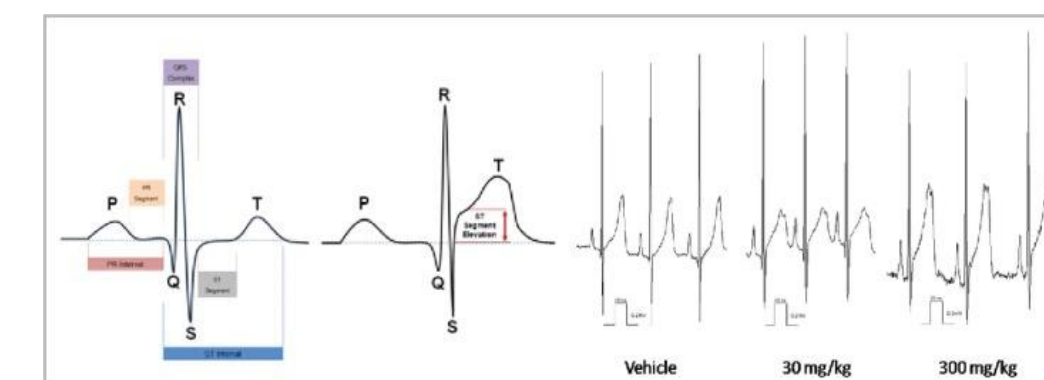
### Literature-based analysis

#### In mice: cardiac structure impaired, function unaffected

- Cleavage of versican defective in the heart of high fat diet-treated *Adamts5*<sup>-/-</sup> 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).

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

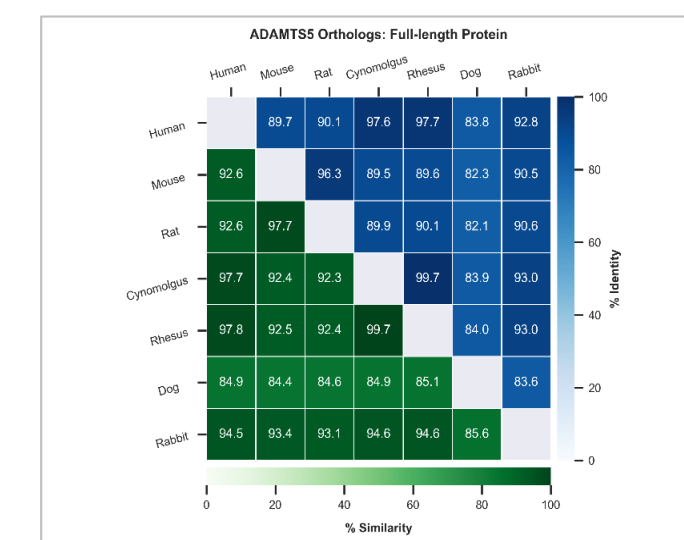
#### In cynomolgus monkey: cardiac function impaired



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

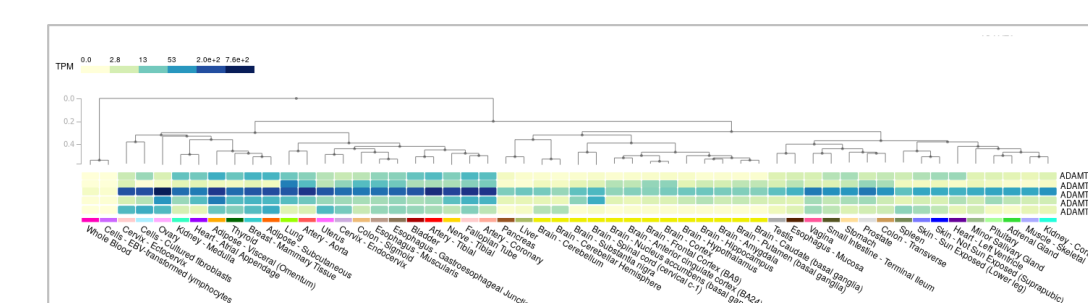
### Informatics analysis

#### 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-ADAMTS4 mAb (GFC301 by Genfrontier)

| Paralogs                        | Epitope sequence |      |     |      |     |      |     |     |     |                  | Percentage conservation across paralogs |
|---------------------------------|------------------|------|-----|------|-----|------|-----|-----|-----|------------------|---|
| ADAMTS4                         | Y <sup>358</sup> | C    | E   | G    | R   | R    | T   | R   | F   | R <sup>368</sup> | 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%              |   |



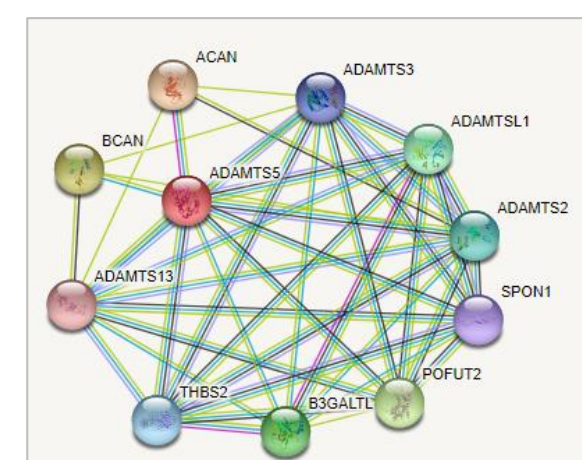
\*ADAMTS4 epitope sequence is used as the base for calculating percentage conservations across paralogs and residue wise conservation.

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

## Target Safety Assessment

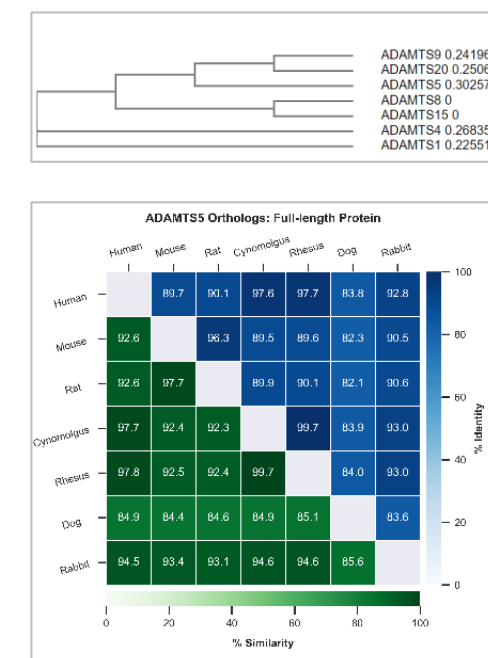
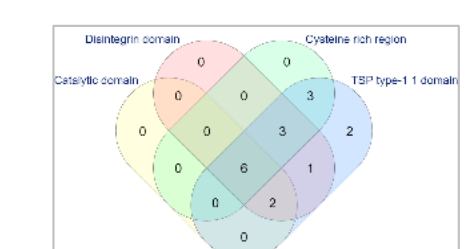
### Target biology in relevance to toxicology

- 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



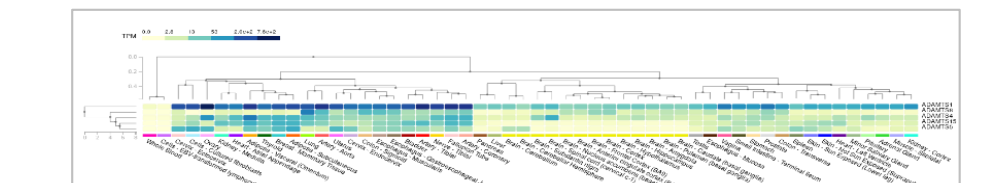
## Target Safety Assessment

## Risk Mitigation Plan

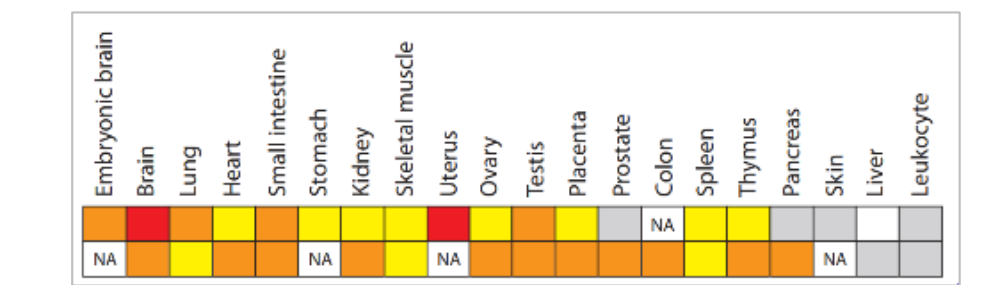
### Polymorphisms/mutations in target

SNPs/mutations associated adverse affects in humans

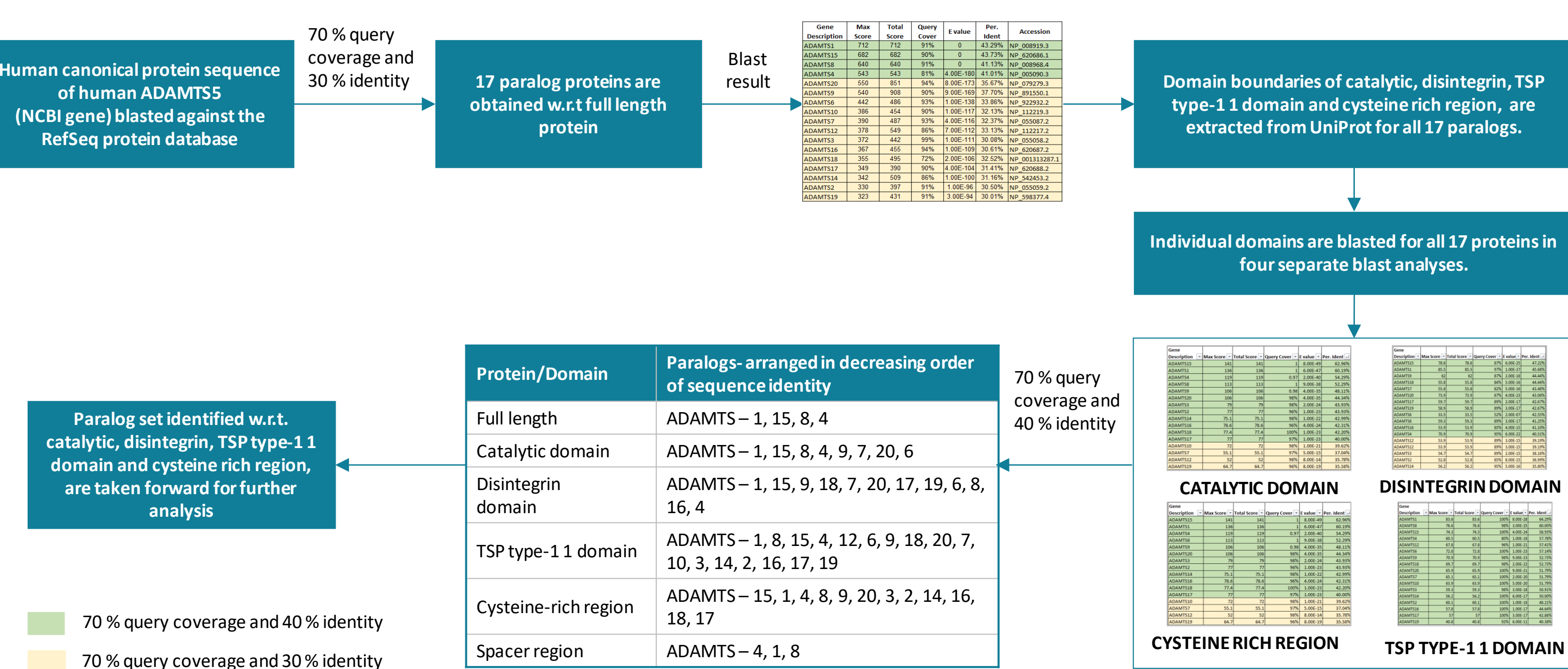
### Toxicities associated with expression



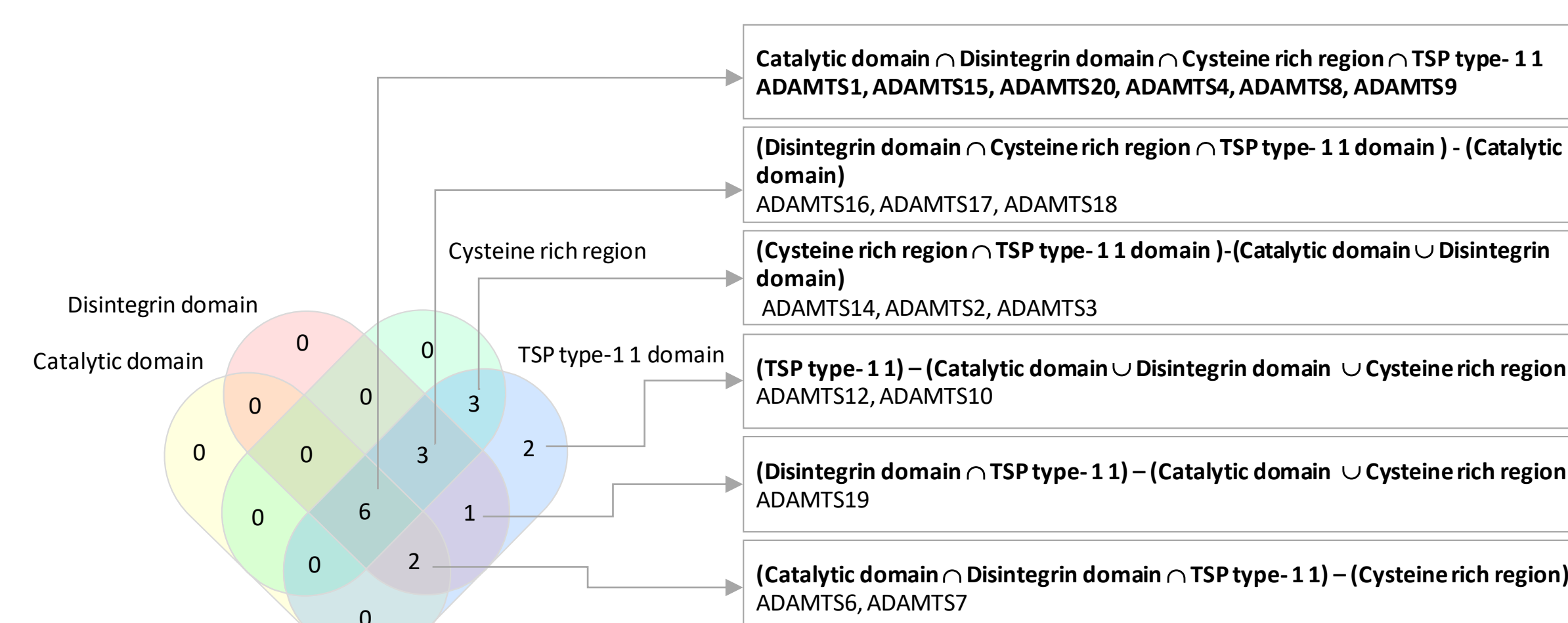
### Safety risks assessment by organ system



## Estimating off- target effects : paralog identification



## Prediction of enhanced efficacy : co-targeting of functional paralogs

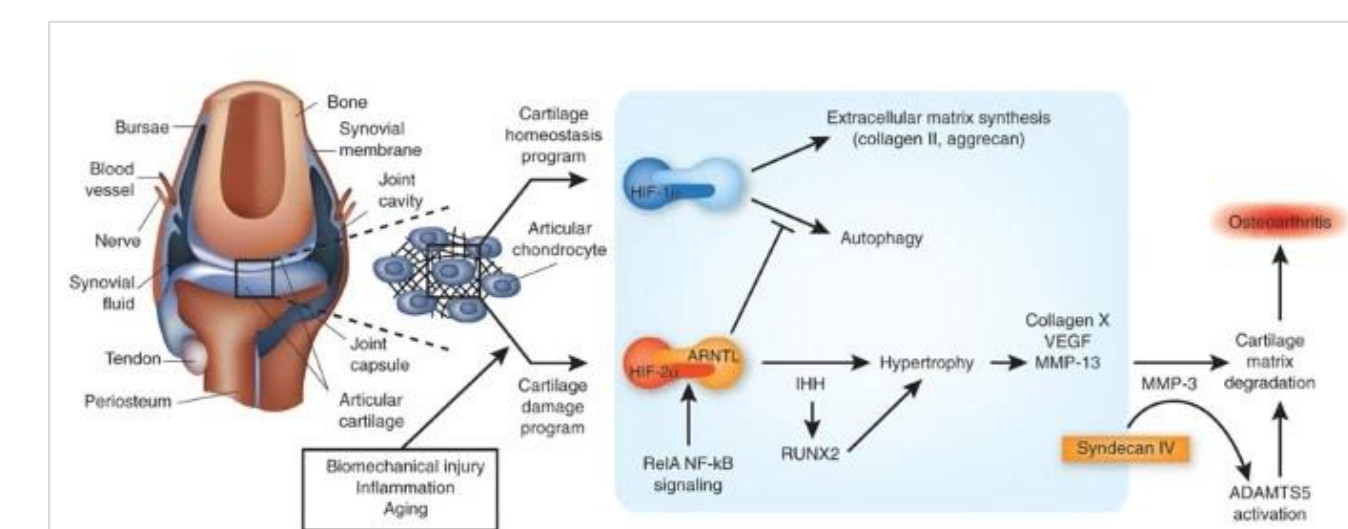


- Common set of paralogs w.r.t. all the domains are ADAMTS1, ADAMTS4, ADAMTS15, ADAMTS8, ADAMTS20 and ADAMTS9.
- Out of these paralogs ADAMTS20 and ADAMTS9 possess lower identities compared to ADAMTS1, ADAMTS4, ADAMTS15 and ADAMTS8.
- ADAMTS4 and ADAMTS5 are the major aggrecanases implicated in arthritis\*.
- 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

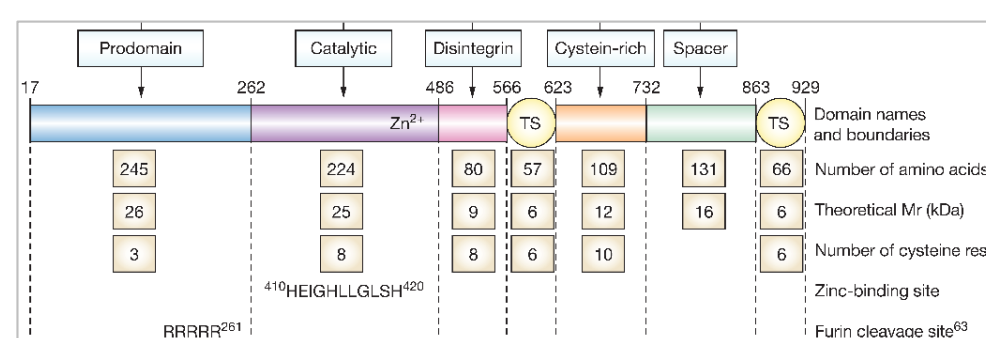
## ADAMTS5 as a target for treating inflammation indications observed in osteoarthritis

- ADAMTS5 (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
- ADAMTS family has 19 members with multiple substrate specificity which defines its range of biological functions



## ADAMTS5 protein domain organisation

- ADAMTS5 is comprised of prodomain, catalytic, disintegrin, TSP-type 1, TSP-type 12 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)

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