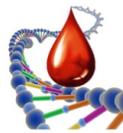


## The Role of von Willebrand Factor in Mediating Factor VIII Immunogenicity

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Milan, March 2016



Clinical and Molecular  
Hemostasis Research Group



### Background

- **Clinical epidemiology studies**
  - SIPPET 1.87-fold increase with rFVIII products
  - CANAL
  - RODIN
  - French Study
- **Experimental/biological studies**
  - to explain why there's a difference in immunogenicity

### General Comments on a Biological Explanation for Differential Immunogenicity

#### Given –

1. Length of time to show difference in immunogenicity (20 yrs +)
2. Immunogenicity difference (1.87-fold), is not binary (yes/no)

#### Biological Explanation will likely be -

- ❖ Relatively subtle
- ❖ Complex

### Why Might Recombinant FVIII be More Immunogenic?

- Increased protein aggregates
- Different post-translational modifications
  - eg, different glycans
- Lacking supplemental plasma immunodulatory proteins
  - eg. TGF $\beta$ , IL-10 etc.
- Reduced VWF binding – limiting an immunomodulatory effect of VWF

### The Factor VIII-VWF Association

FVIII	VWF
Endothelial cells	Endothelial cells Megakaryocytes
200 ngs/mL	10 µg/mL
1 nM	35 nM

### The Factor VIII – VWF Association

Kd - 0.2 nmol/L

At saturation: Stoichiometry 1 FVIII: 50 VWF



The affinity and stoichiometry of binding of human factor VIII to von Willebrand factor. Vlot et al. Blood. 1995

## Established VWF-FVIII Associations

- Synthesized together in some endothelial cells
- VWF influence dominant on FVIII clearance

## Presentation Summary

### 1. Brief review of 5 experimental reports

Pfistershammer et al. Thromb Haemost 2006

Qadura et al. Blood 2009

Dasgupta et al Blood 2007/Delignat et al Haemophilia 2012

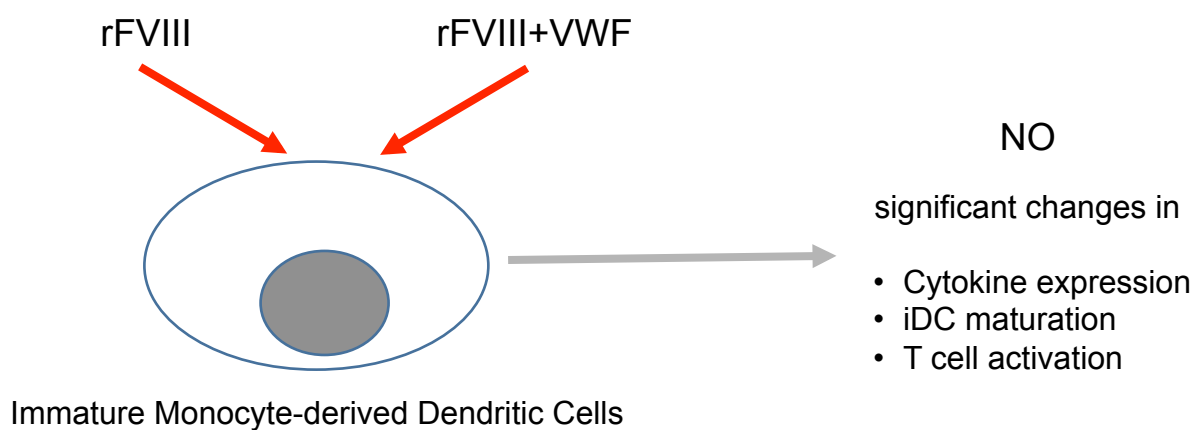
Sorvillo et al Haematologica 2015

### 2. Proposal of a novel hypothesis implicating VWF as an immunomodulatory influence for FVIII inhibitor development

Recombinant factor VIII and factor VIII von Willebrand factor complex  
do not present danger signals for human dendritic cells

Pfistershammer et al. Thromb Haemost. 2006

Neither rFVIII nor rFVIII-VWF complexes result in Immature Dendritic Cell Activation



Pfistershammer et al. Thromb Haemost. 2006

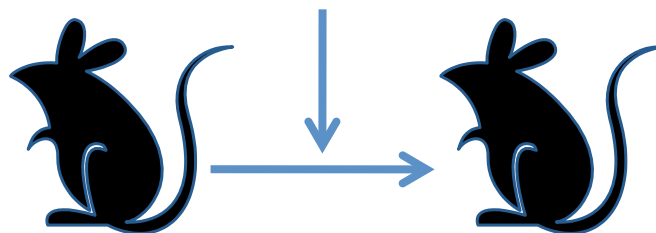
Recombinant and plasma-derived factor VIII products induce distinct splenic cytokine environments in hemophilia A mice

Qadura M et al. Blood. 2009.

Factor VIII “Immunization” Protocol

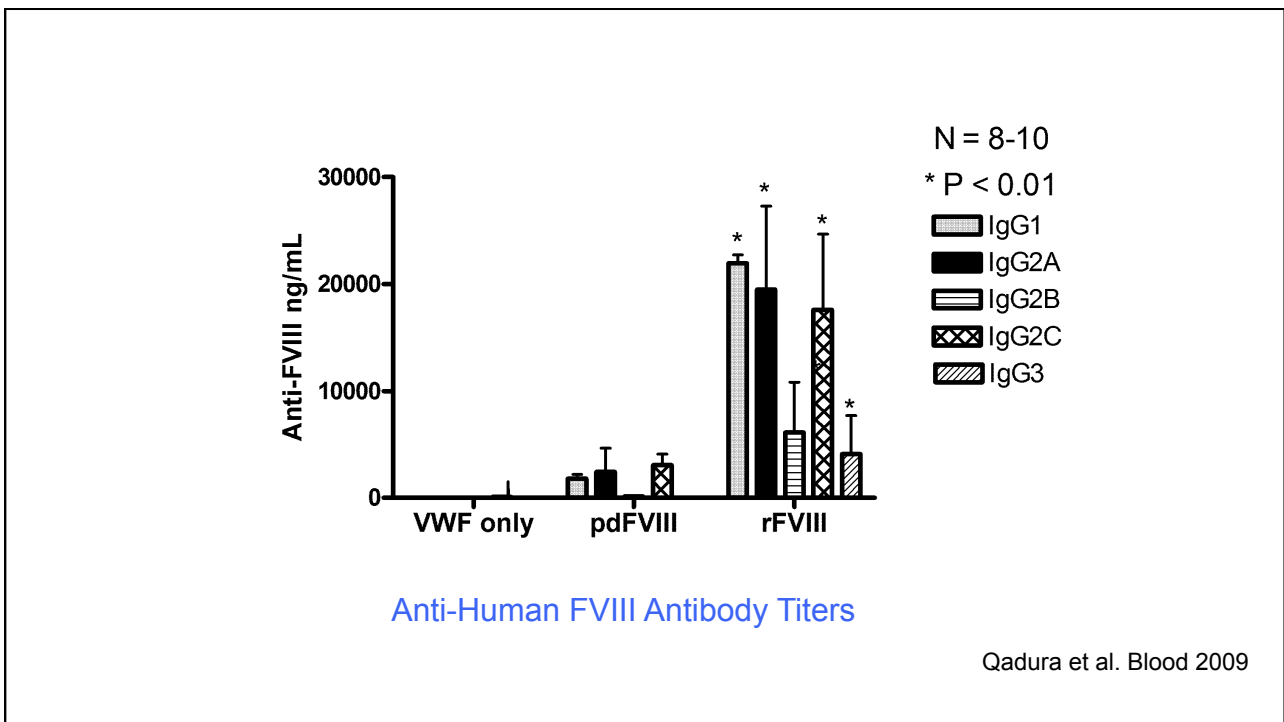
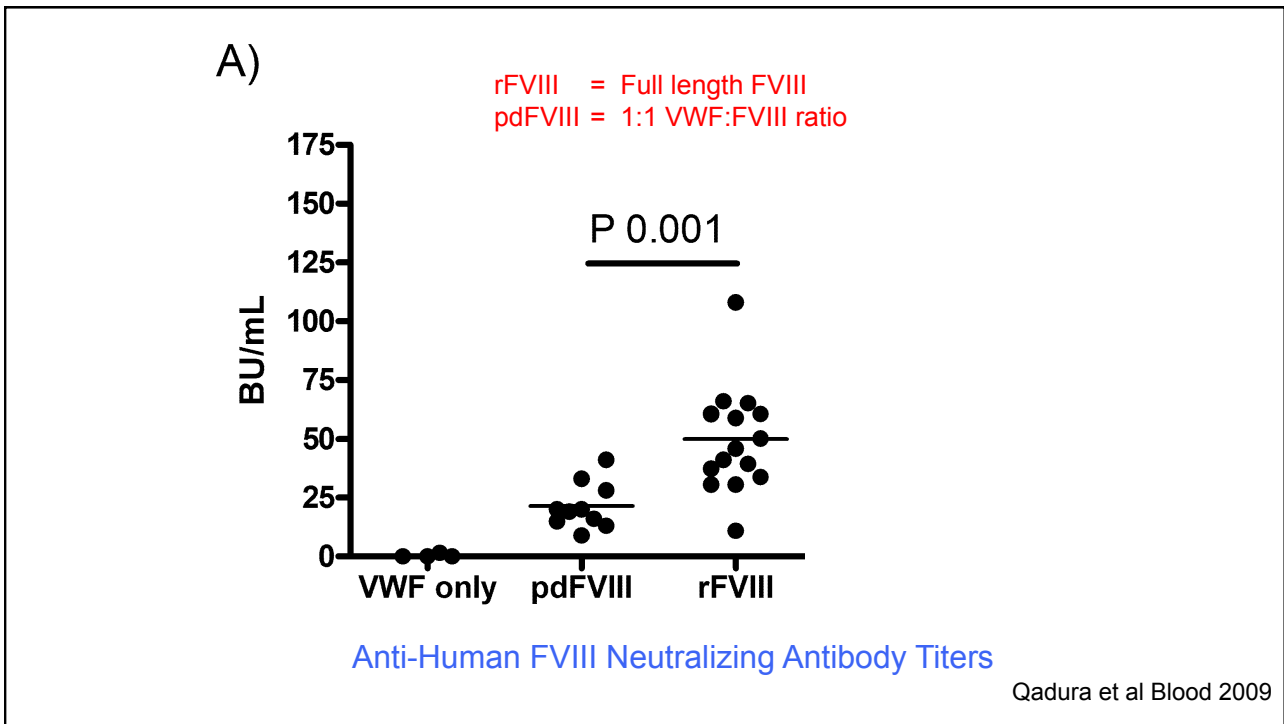
4 weekly IV infusions

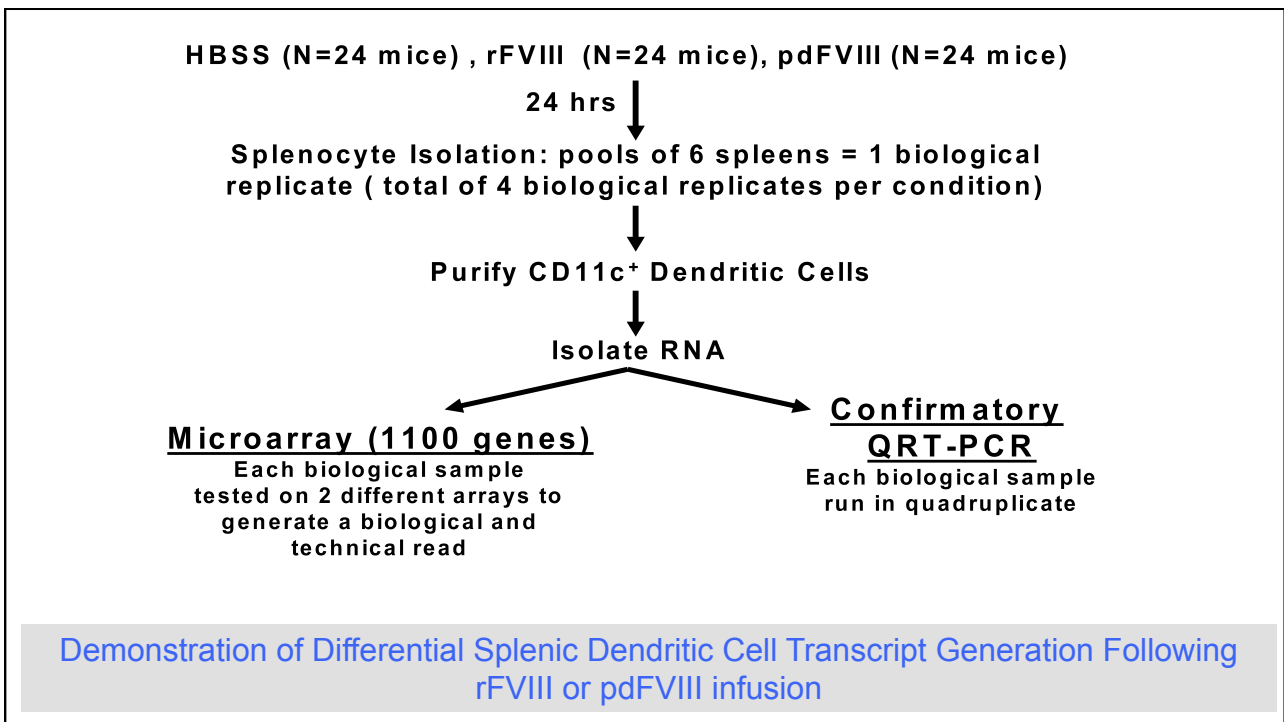
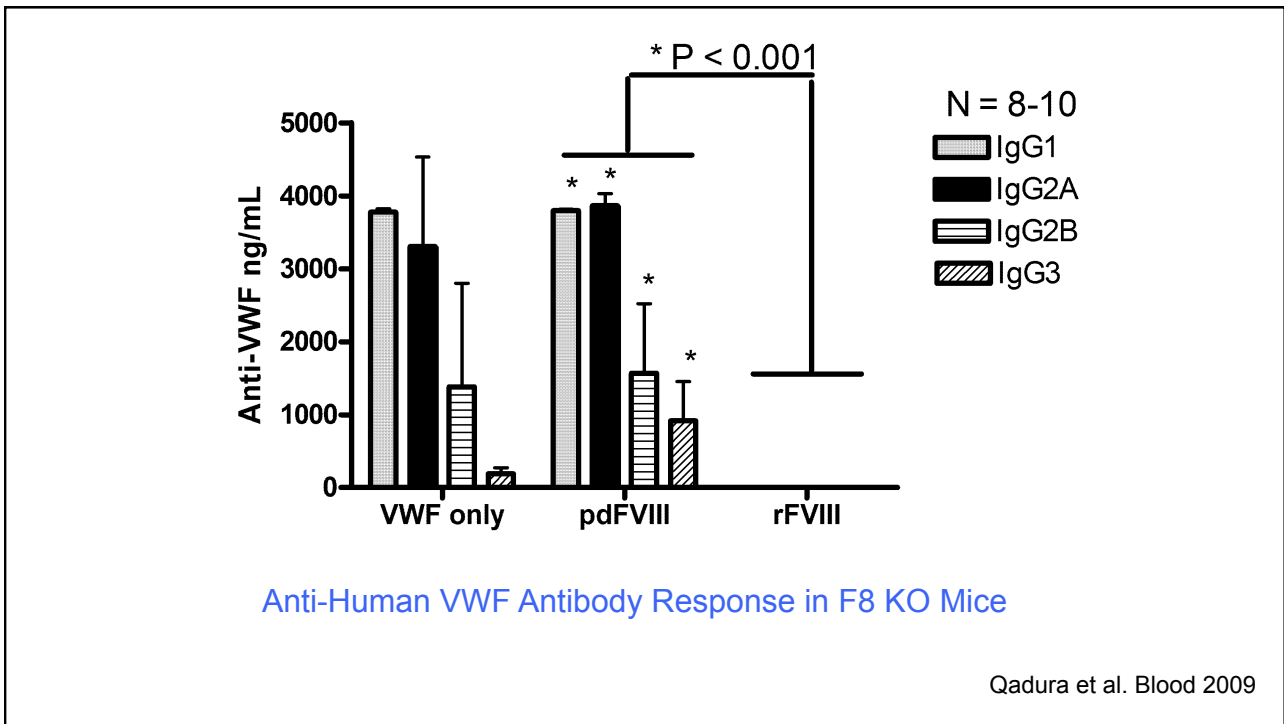
2 IU (80 U/kg: ~400 ngs) FVIII



1 week later, test for anti-FVIII immune response

Qadura M et al. Blood. 2009.







### Differential Immune Response Gene Expression

Gene Name	rFVIII/pdFVIII Ratio
Ccl2	0.25
Cxcl1	0.39
Cxcl2	0.41
Hspb1	0.44
Hspa1a	0.45
Il6	0.46
Jun	0.47
Gdf15	0.47
Egr1	0.47
Plk2	0.48
Egr2	0.49
Itga2b	2.01
Cxcl4	2.10
Ltf	2.17
Ppbb	2.41
Camp	2.72

#### Consistent 2-fold difference

4 biological replicates  
6 technical replicates  
qRT-PCR x 4

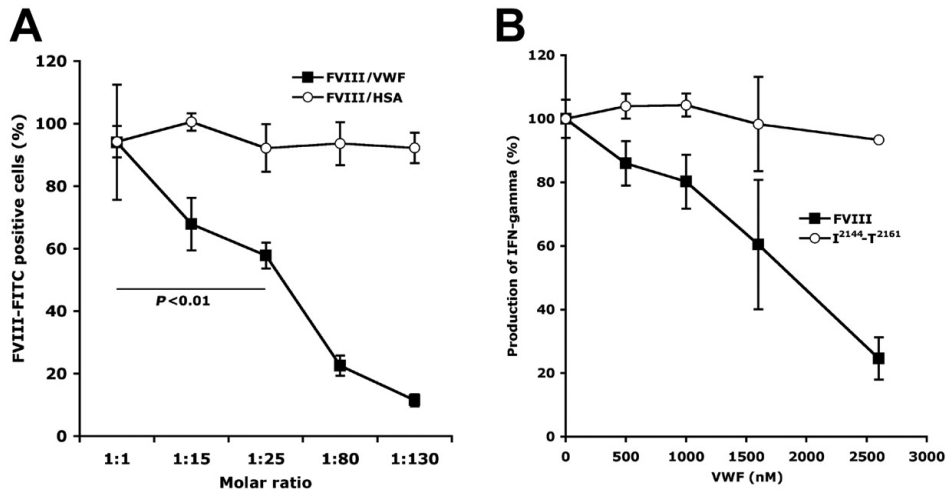
16 immune response transcripts

- Ccl2 – chemokine ligand 2
- Heat shock protein 1
- Lactotransferrin

VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors

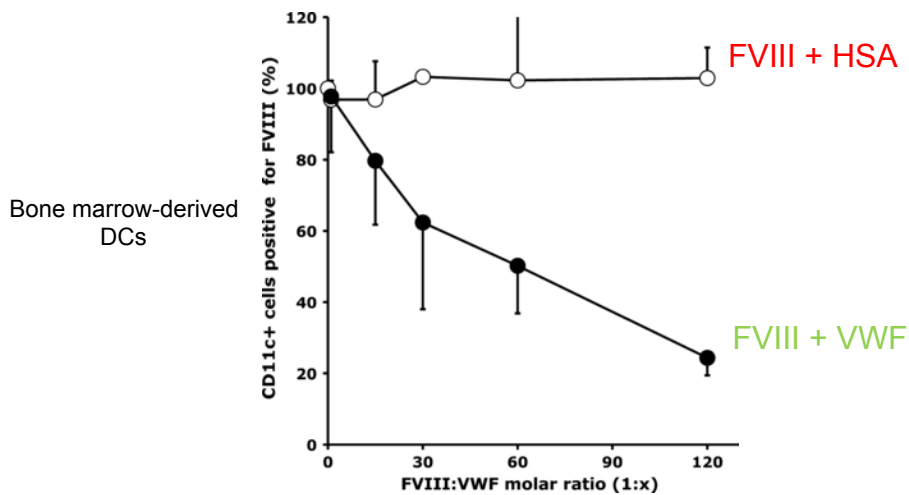
Dasgupta et al Blood 2007

VWF reduces FVIII endocytosis by DCs and the consequent presentation to FVIII-specific T cells



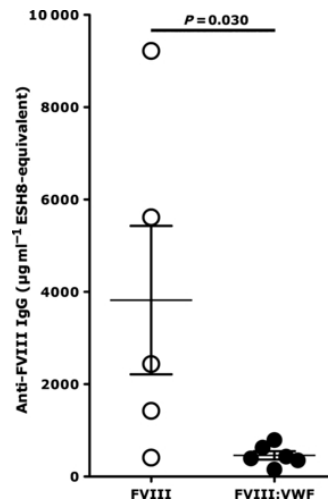
Dasgupta et al Blood 2007

Immunoprotective effect of von Willebrand factor towards therapeutic factor VIII in experimental haemophilia A



Haemophilia 18: 248-254, 2012

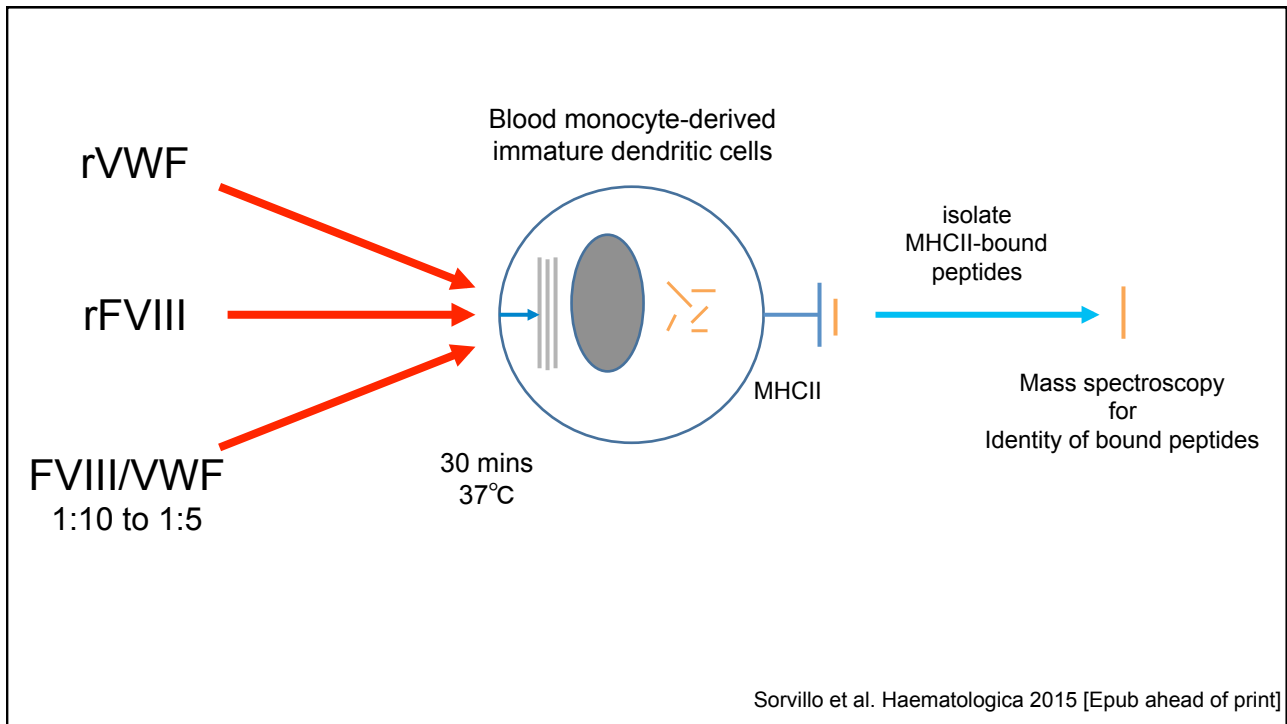
Levels of anti-FVIII IgG in F8 KO mice after injection of FVIII (empty circles) or FVIII with a 50-molar excess of purified VWF (filled circles)



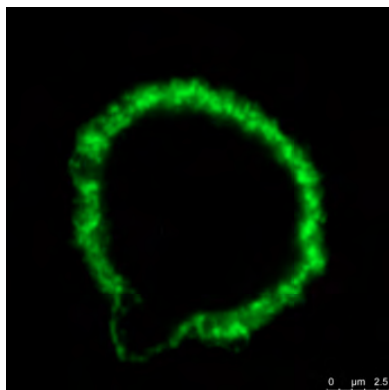
Delignat et al Haemophilia 2012

## Von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII

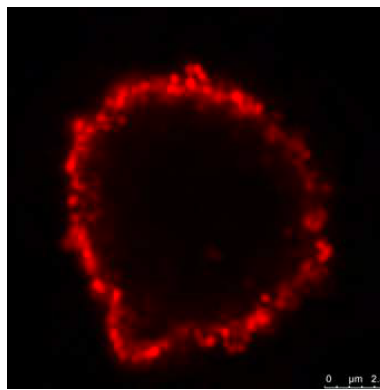
Nicoletta Sorvillo et al. Haematologica 2015 [Epub ahead of print]



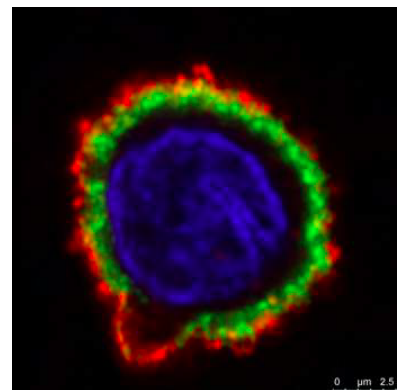
### Binding of VWF to iDCs assessed by confocal microscopy.



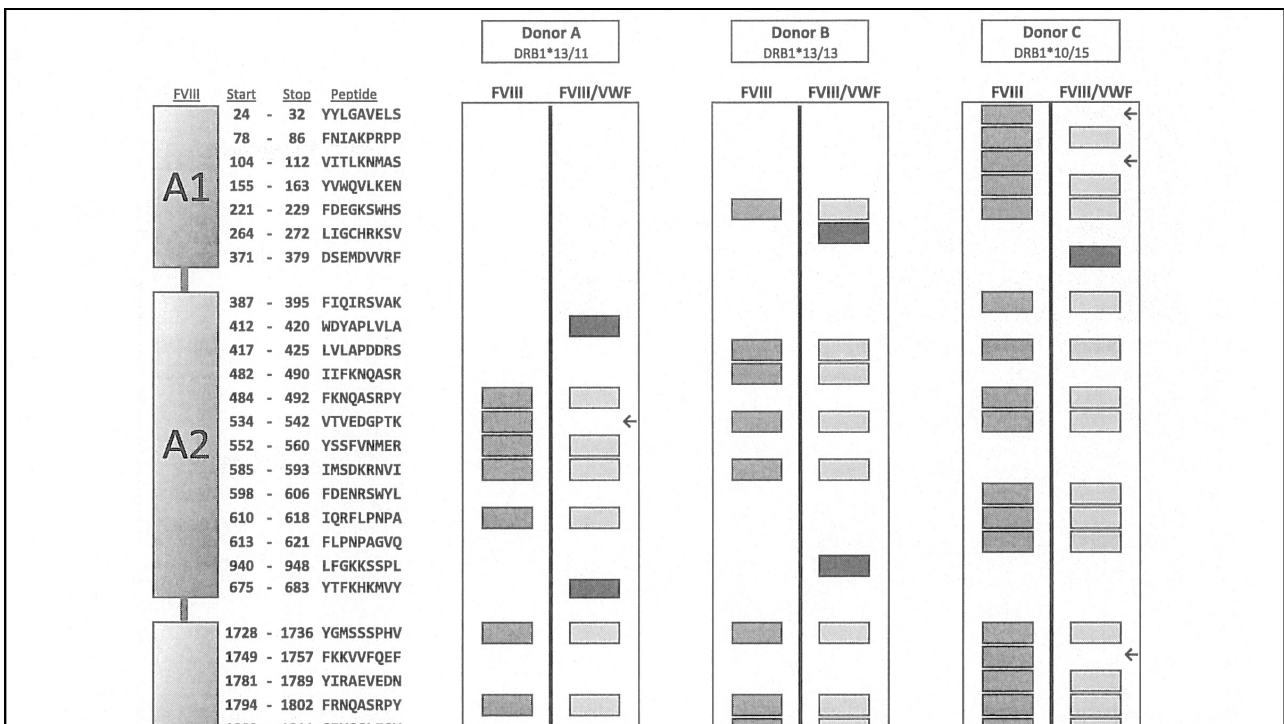
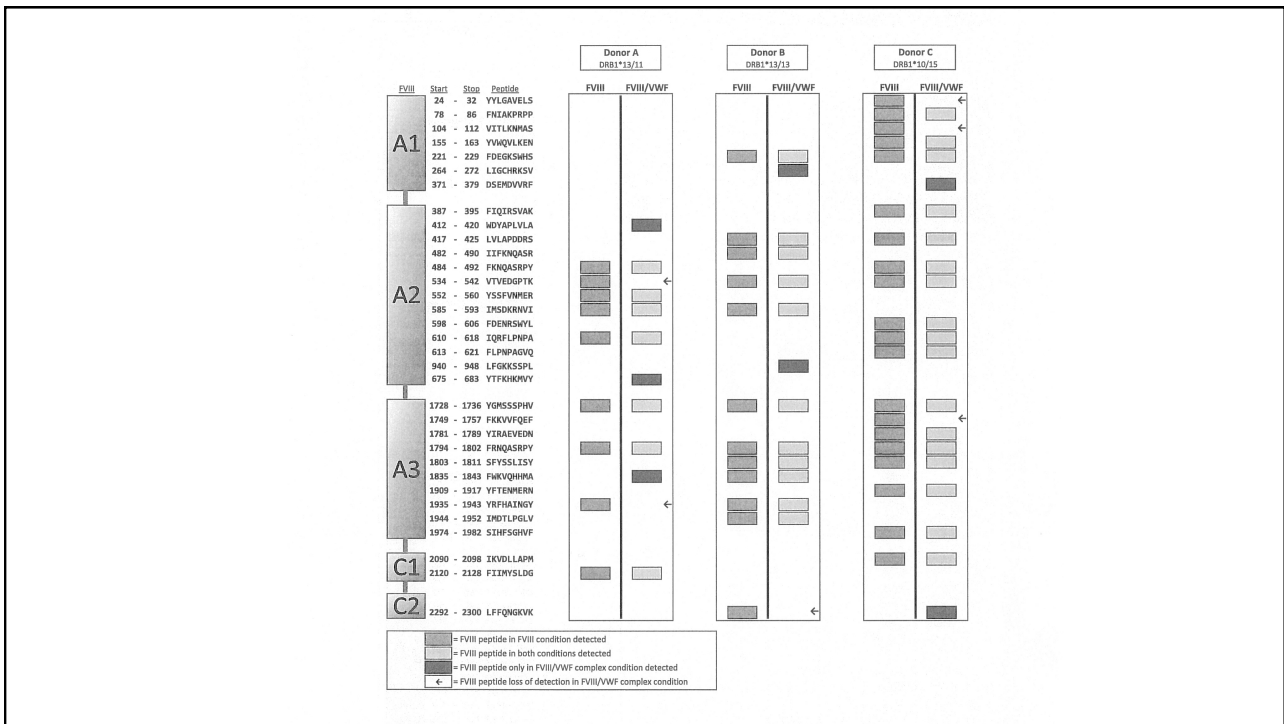
DC-SIGN

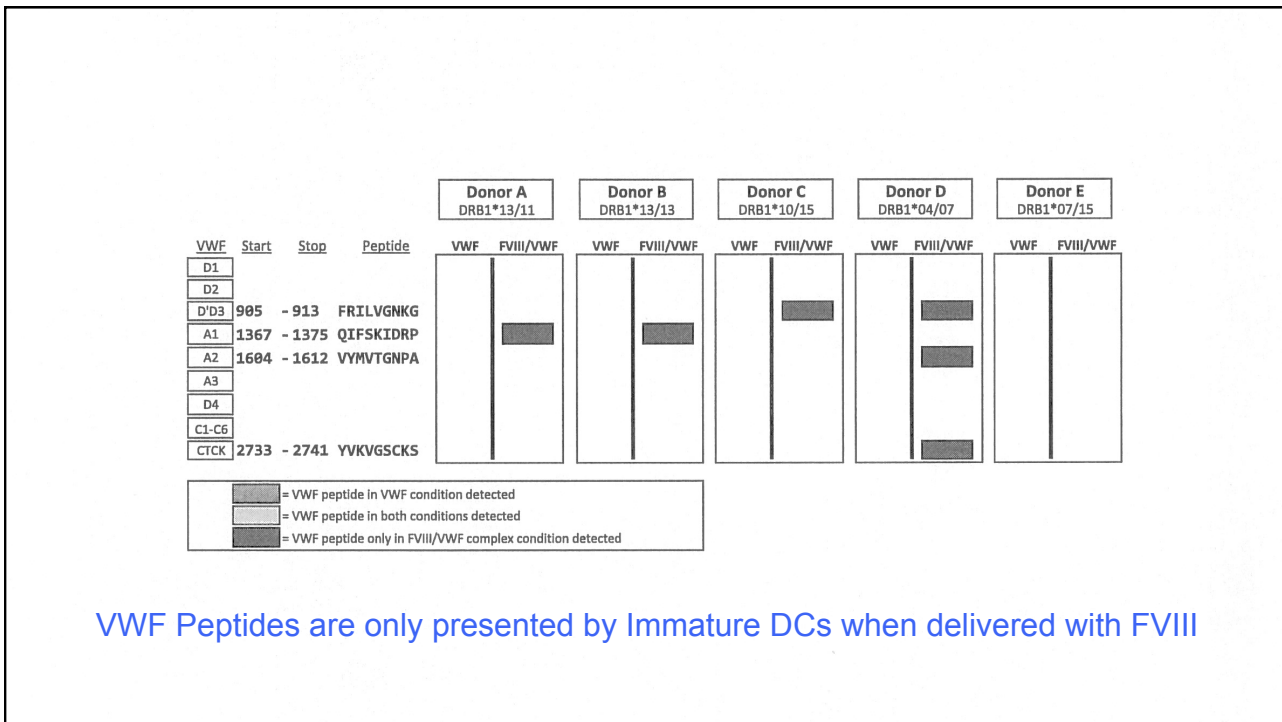


VWF



Merge





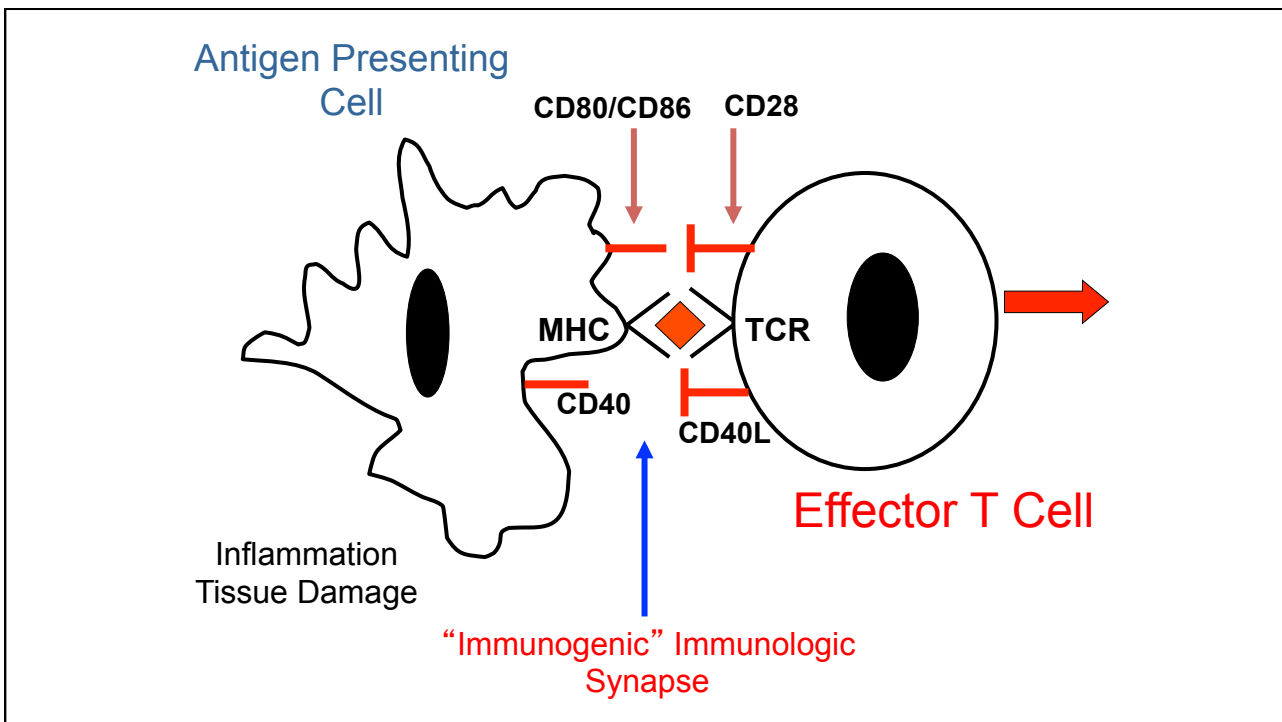
## Von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII

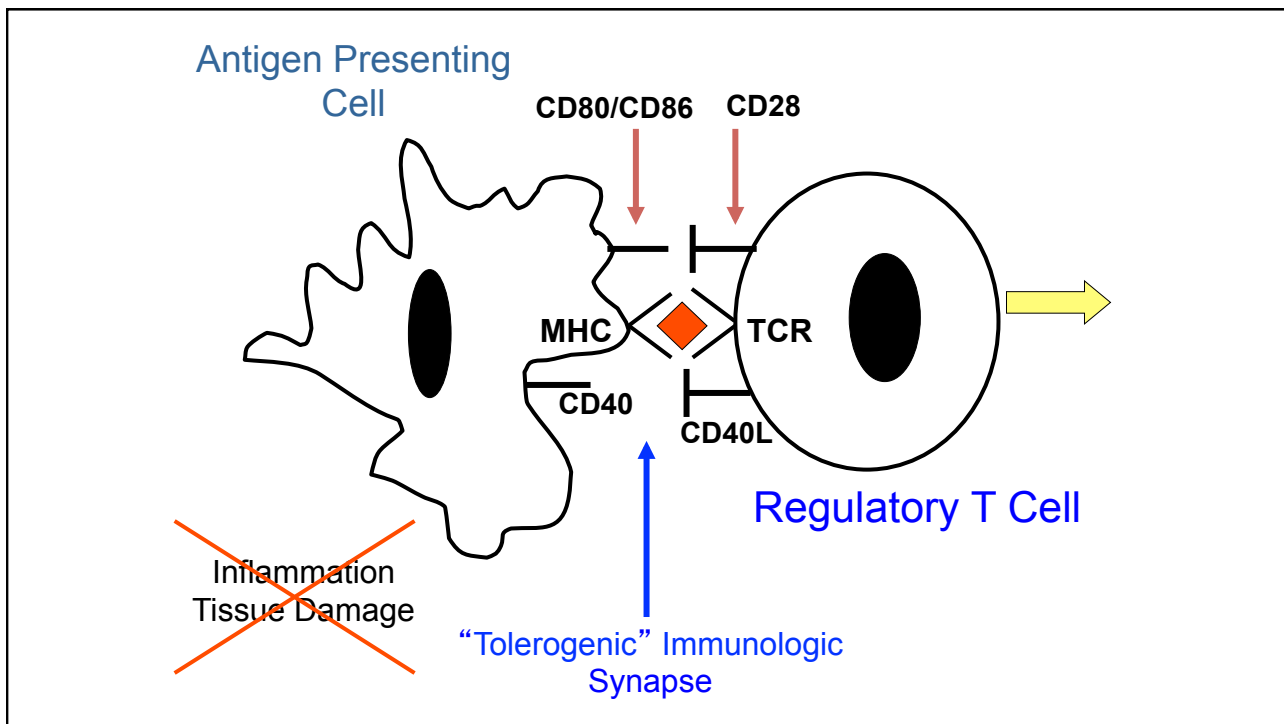
### Conclusions

- VWF binds to the surface of immature DCs but is not internalized
- VWF reduces uptake of FVIII by immature DCs
- Different FVIII peptides were presented with VWF/FVIII vs FVIII alone
- VWF requires FVIII for the presentation of any VWF peptides by immature DCs

## Questions Posed by the Reported Experimental Observations

1. If FVIII presentation is inhibited by VWF, how is FVIII tolerance generated?
2. Are monocyte-derived immature DCs a good candidate for FVIII antigen presentation?
3. Why doesn't the patient's own intrinsic VWF fulfill these functions with rFVIII?



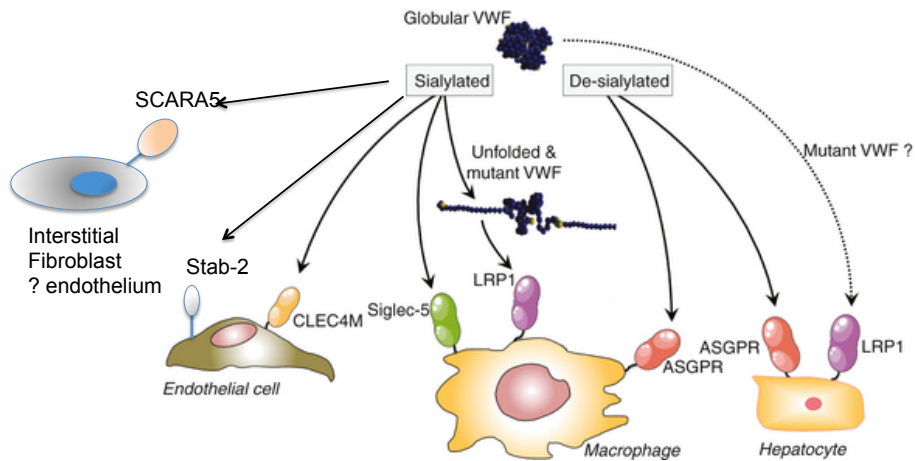


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## Clearance Receptors for the VWF-FVIII



Derived from (Casari et al. 2013)

## What are the Antigen Presenting Cells for VWF–VWF?

### Candidate antigen presenting cells for VWF-FVIII

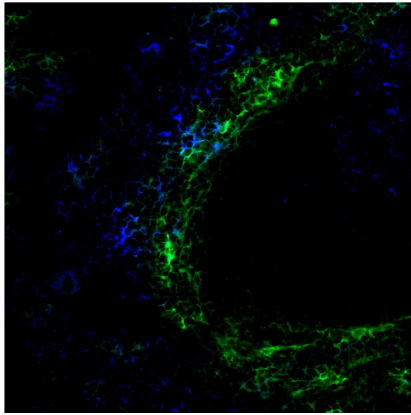
#### ▪ Spleen

- ❖ Marginal zone macrophages
- ❖ Metallophilic macrophages
- ❖ Marginal zone B cells
- ❖ Dendritic cells

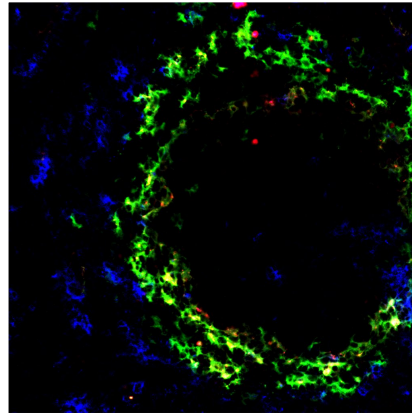
#### ▪ Liver

- ❖ Kupffer cells
- ❖ Dendritic cells
- ❖ Sinusoidal endothelial cells

### Co-Localization of FVIII with Splenic Marginal Zone Macrophages (MARCO +ve)



PBS Infusion

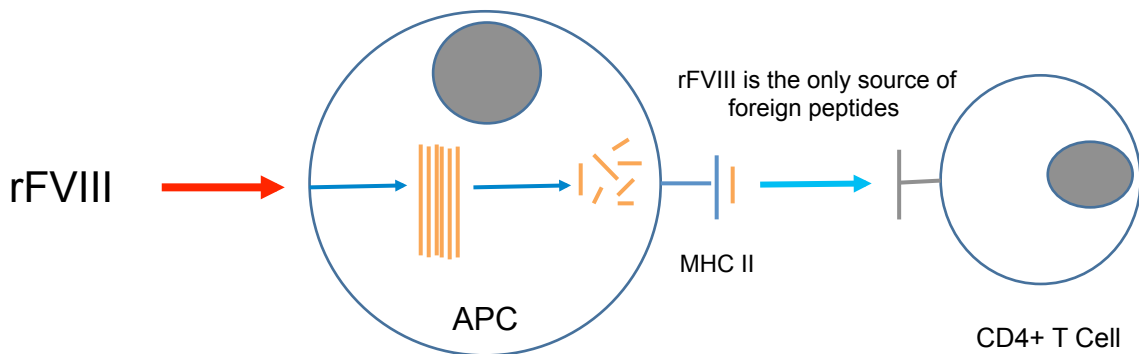


30 mins post-FVIII Infusion

Does the absence of VWF change this localization?

Data representative of at least 2 biological replicates  
Jesse Lai, Unpublished data

### rFVIII Presentation in a naïve Hemophilia A Patient



Tolerance already established for intrinsic (self) VWF and other plasma proteins

No competition for rFVIII Immunogenicity

### Contrast with pdFVIII-VWF and Naïve PUP Responses

pd-FVIII - Variably tolerant

pd-VWF - have "self" VWF – therefore tolerant?

Many other plasma proteins - have "self" proteins therefore tolerant

### Heterogeneity of pdVWF-FVIII

However,

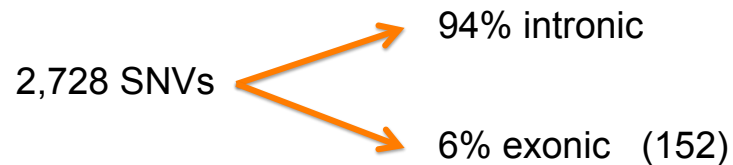
pdVWF and FVIII are polymorphic proteins

and

pdFVIII concentrates derive from 1,000s of donors

VWF Genomic Variability in 1000 Genomes

(Wang et al. JTH 2013)



91 of 152 exonic SNPs - non-synonymous (AA substitutions)

Minor allele frequencies: 0.05 – 3.3%  
(but pdFVIII lots ~>10,000 donors)

Complexity and diversity of F8 genetic variations in the 1000 genomes

(Li et al. JTH 2015)

3,030 SNVs

2.18% exonic (85/3,030)

56/85 variants are missense substitutions

Again, minor allele frequencies are low

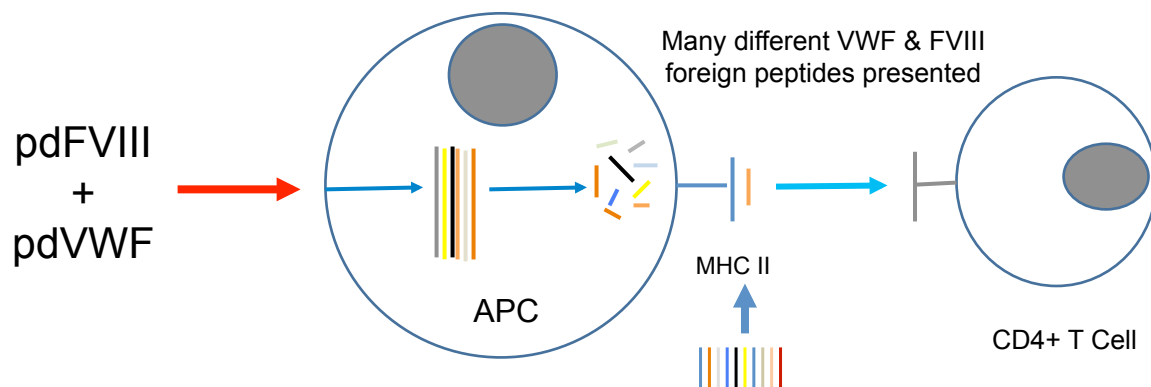
## Heterogeneity of pdFVIII Concentrates

Therefore ....

Infusion of pdFVIII involves exposure to

91 variant forms of VWF  
&  
56 variant forms of FVIII

## pdFVIII Presentation in a naïve Hemophilia A Patient



FVIII immunogenicity is "distracted"/"diluted" by antigen competition from many pdFVIII-derived VWF and FVIII variant proteins

## Conclusion

There is increasing evidence that the immune response to rFVIII is different to that for pdFVIII.

However, this difference has only emerged after >25 years of clinical use of rFVIII, and there is still not consensus about the significance of these recent findings.

In light of the importance of FVIII inhibitor development, further studies of the epidemiology of FVIII immunogenicity are required to confirm recent reports, and basic science experiments are needed to provide a biological basis that would support an apparent differential immunogenicity associated with rFVIII



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