



# Understanding the mechanism of TTS – work sponsored by AstraZeneca

Taylor Cohen, AstraZeneca

June 27<sup>th</sup> 2022

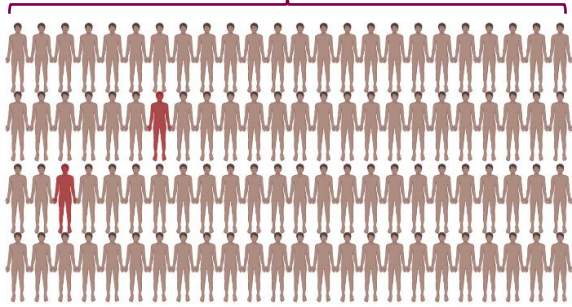
Strictly confidential


Classified as internal/staff & contractors by the European Medicines Agency



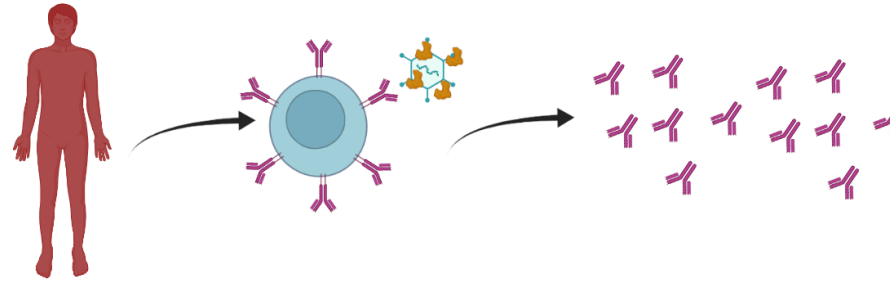
# Hypothesized Mechanism Leading to TTS



AZD1222 Vaccine Recipients < 2% Express anti-PF4 IgG who might be at risk of TTS





 Individual with pre-existing B-cells encoding anti-PF4 IgG  
(not all anti-PF4 is pathogenic)

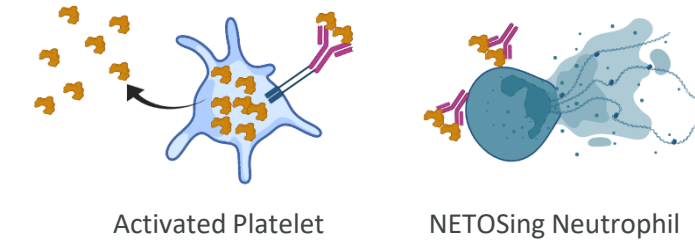
Individuals with B-cells encoding pathogenic anti-PF4 are boosted by ChAdOx1-PF4 complex



 B-cells encoding anti-PF4 IgG  
 ChAdOx1

 anti-PF4 IgG  
 PF4

Pathogenic anti-PF4 IgG Triggers Thrombosis via Activation of Platelets and Neutrophils



Activated Platelet

NETOSing Neutrophil

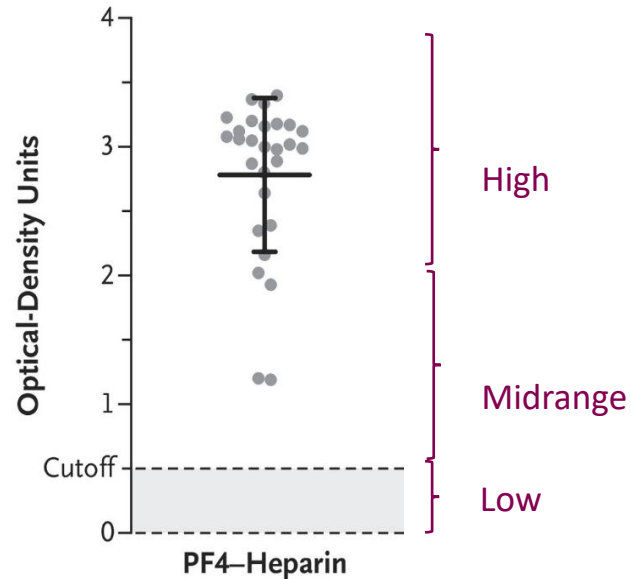
**Hypothesis:** ChAdOx1-PF4 complexes are recognized by B-cells encoding pathogenic anti-PF4 IgG, boosting production of TTS inducing anti-PF4 IgG and initiating thrombosis

## Key Pieces of Evidence:

- Presence of low level anti-PF4 and platelet activation does not predict TTS
  - ~2% of individuals are positive for low levels of anti-PF4 IgG and did not develop TTS post vaccination
- AZD1222 does **not** increase anti-PF4 levels in healthy vaccine recipients
- IgG from TTS patients is capable of activating platelets and neutrophils, and inducing thrombosis in a murine model (hFcyRIIIa/hPF4)

# AZD1222 (ChAdOx1 nCoV-19) did not increase anti-PF4 antibody levels in US/Chile/Peru Phase 3 study

## ELISA Determination of anti-PF4 Levels in TTS Patients



Greinacher, A. et al. N Engl J Med. 2021;384:2092–2101

## AstraZeneca US/LatAm Ph3 Data – anti-PF4 Levels

~2,600 participants at two timepoints

### US/LatAm Phase 3 Substudy

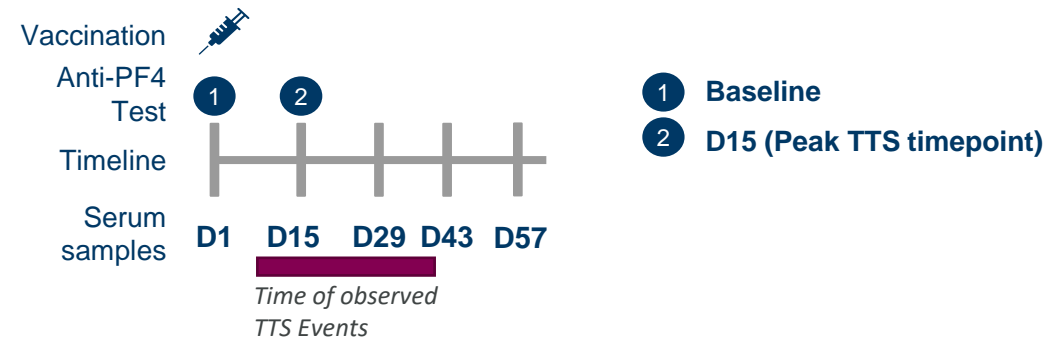


Table 1. Changes in Anti-PF4 Antibody Levels Between Baseline, Prior to Administration of AZD1222 or Placebo, and Day 15\*

Treatment group	Level	Baseline n (%)	Day 15			p-value†
			Low	Moderate	High	
AZD1222	Low	1,727 (98.0)	1708 (98.9)	19 (1.1)	0	
	Moderate	35 (2.0)	10 (28.6)	25 (71.4)	0	
	High	0 (0)	0	0	0	
	<b>Total</b>	<b>1,762 (100)</b>	<b>1718 (97.5)</b>	<b>44 (2.5)</b>	<b>0</b>	
Placebo	Low	857 (97.7)	850 (99.2)	7 (0.8)	0	
	Moderate	20 (2.3)	6 (30.0)	13 (65.0)	1 (5.0)	
	High	0 (0)	0	0	0	
	<b>Total</b>	<b>877 (100)</b>	<b>856 (97.6)</b>	<b>20 (2.3)</b>	<b>1 (0.1)</b>	

AZD1222 vs placebo: proportion changing from 'low' (baseline) to 'moderate' or 'high' (Day 15) p=0.676

### Interpretation:

- No statistical difference in shift to abnormal range between placebo and AZD1222 (p=0.674)
- Minimal shift observed between baseline and Day 15
- Limited by small sample size, and does not reflect avidity of antibodies

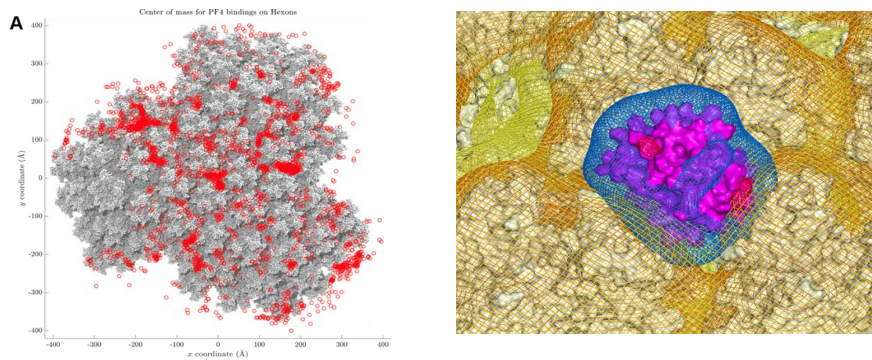
Cohen T, et al. Sci Rep. 2022;12(1):7961.



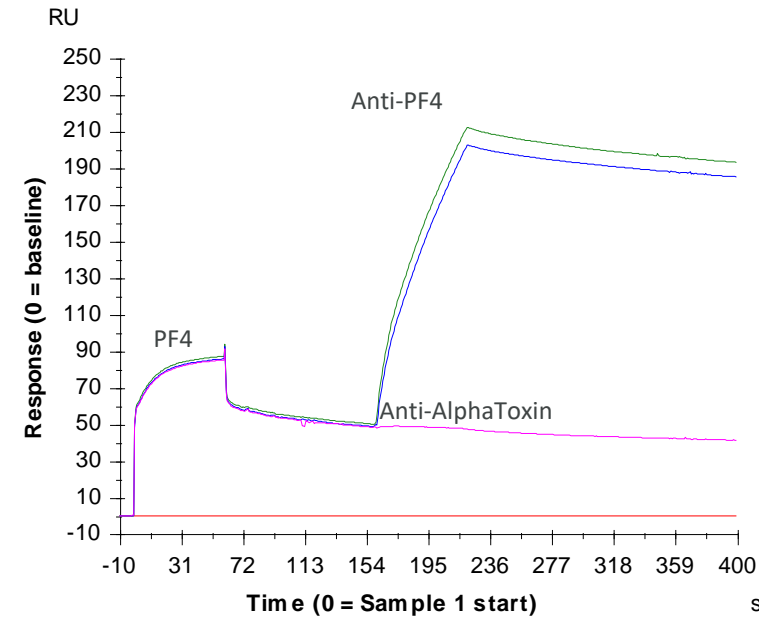
# Binding of PF4 to ChAdOx1 Observed *in vitro*

**Hypothesized mechanism:** PF4/ChAdOx1 complex is recognized by anti-PF4 IgG, activating platelets via FcR binding

**Computational prediction of PF4-ChAdOx1 interaction.**



**PF4-ChAdOx1 Binding Demonstrated by SPR**



- Binding is charge dependent (High NaCl blocks)
- Also observed with other adenoviruses (Ad26, Ad5)

## Next steps/Interpretation:

- Confirms one step of potential HIT like mechanism (PF4 is able to bind ChAdOx1 *in vitro*)
- **Question:** Does PF4 from TTS patients and healthy donors differ?
- **Question:** Does ChAdOx1/PF4 activate B-cells?

Baker AT, et al. Sci Adv. 2021;7(49):eabl8213.

