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Foundation for Anemia Research

Innovative drug design using RNA aptamers for various anemias

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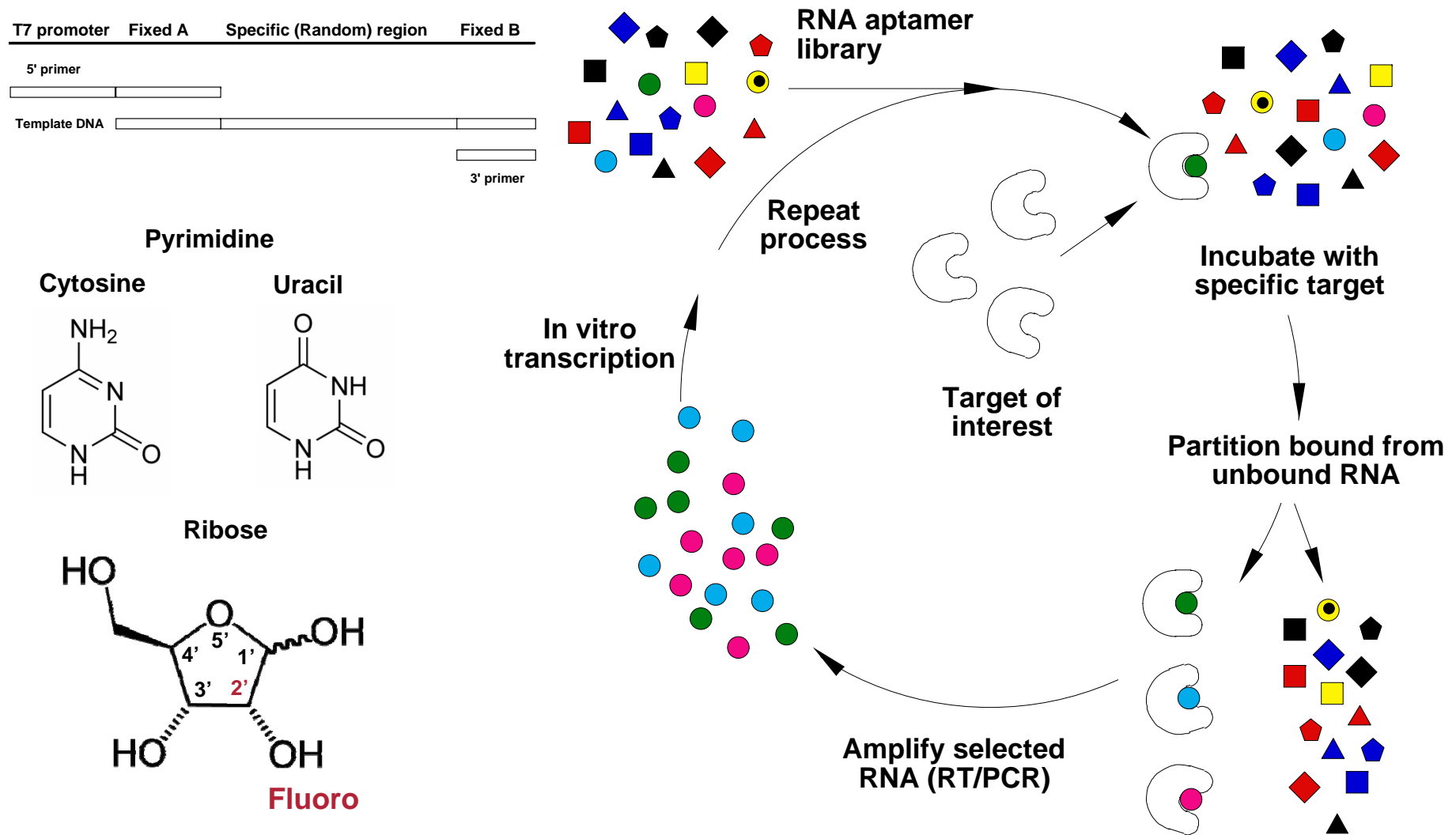
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RNA Aptamers

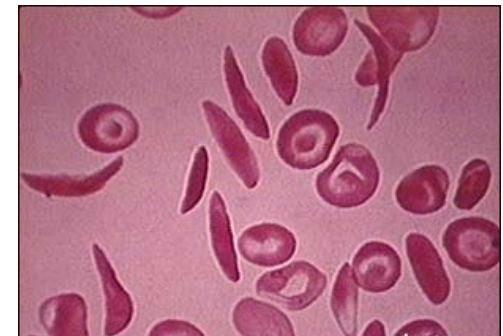
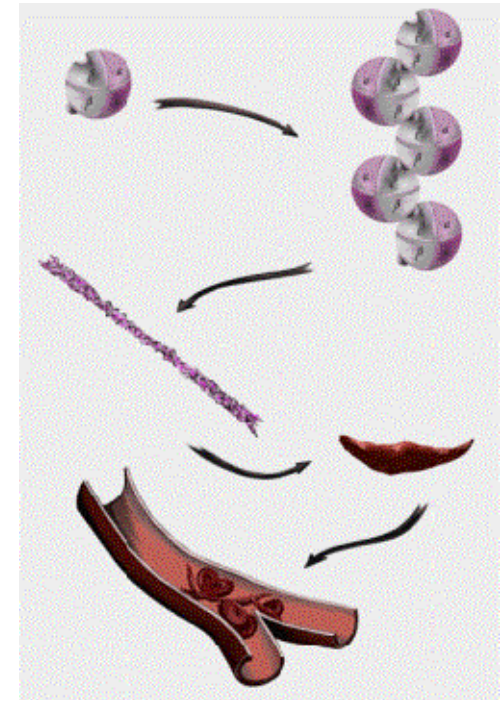
- Oligonucleotides that **bind to specific ligands** are termed "**aptamers**" (from the Latin word 'aptus', to fit) by Ellington and Szostak (1990).
- Selection process was named **SELEX** (**s**ystematic **e**volution of **l**igands by **e**xponential enrichment) by Tuerk and Gold (1990).
- Aptamers, functionally and conceptually similar to monoclonal antibodies, can directly inhibit a protein's function by folding into specific three-dimensional structures that provide high-affinity binding to the target protein.
- The first aptamer drug (Macugen), an anti-VEGF aptamer for the treatment of age-related macular degeneration (AMD), has been approved by the US FDA, and additional aptamers are in the clinical pipeline.

An Overview of the SELEX Process



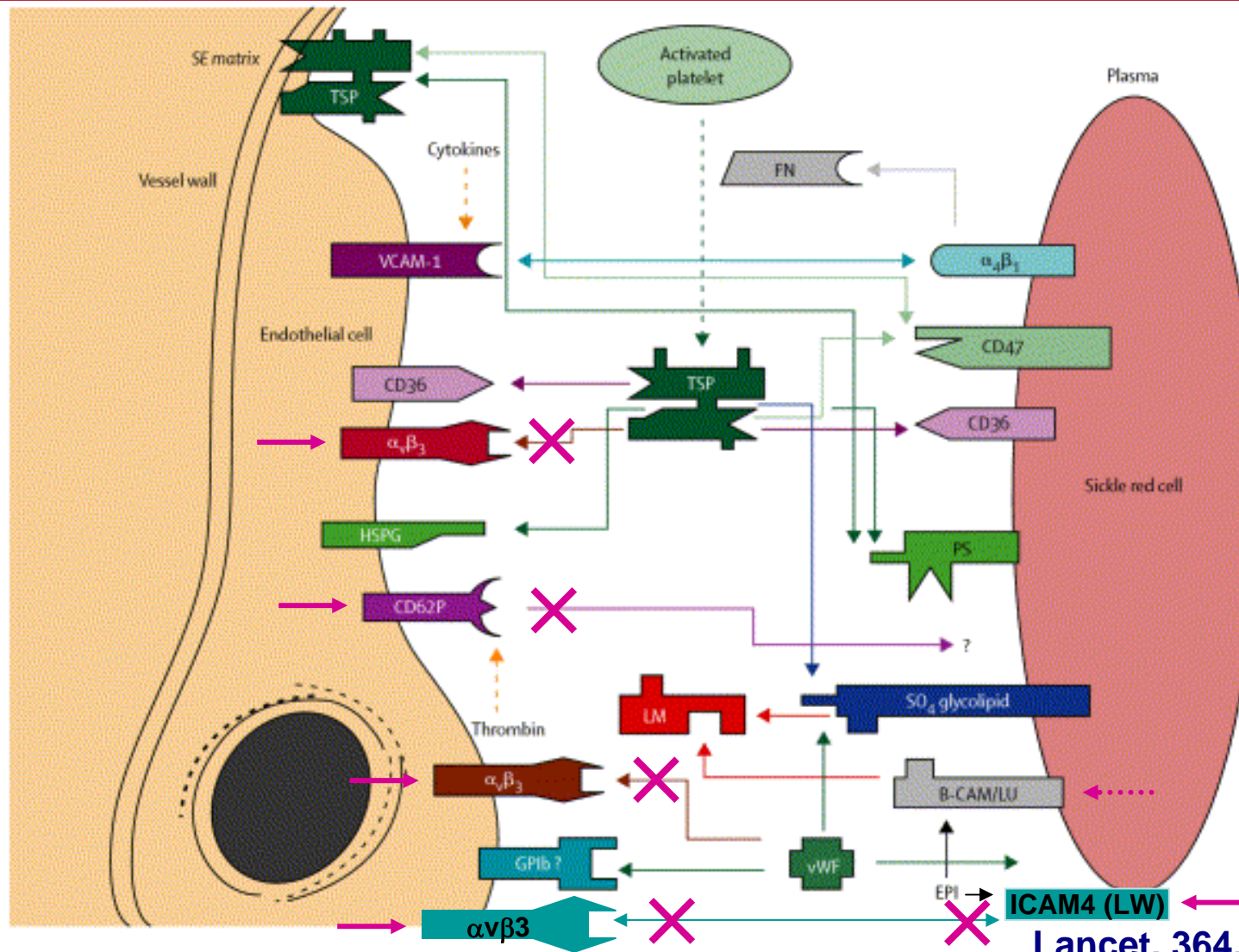
Vaso-Occlusion in Sickle Cell Disease (SCD)

- Most common single gene disorder (β^6 , Glu→Val) in black Americans (1 in 375 affected). Amino acid substitution changes the β chain and its interactions with other β chains, leading to polymerization.
- Entrapment of sickle cells in the microcirculation leads to tissue ischemia and damage in almost all organs. This vaso-occlusive process accounts for the majority of SCD-related complications, including painful crisis, abdominal crisis, hand-foot syndrome, acute chest syndrome, stroke, renal dysfunction, and skin ulceration.
- One contributor to vaso-occlusion is believed to be adhesive interactions between receptors on sickle erythrocytes and endothelial cells, as well as extracellular matrix components.



<http://rad.usuhs.mil/sickle/>

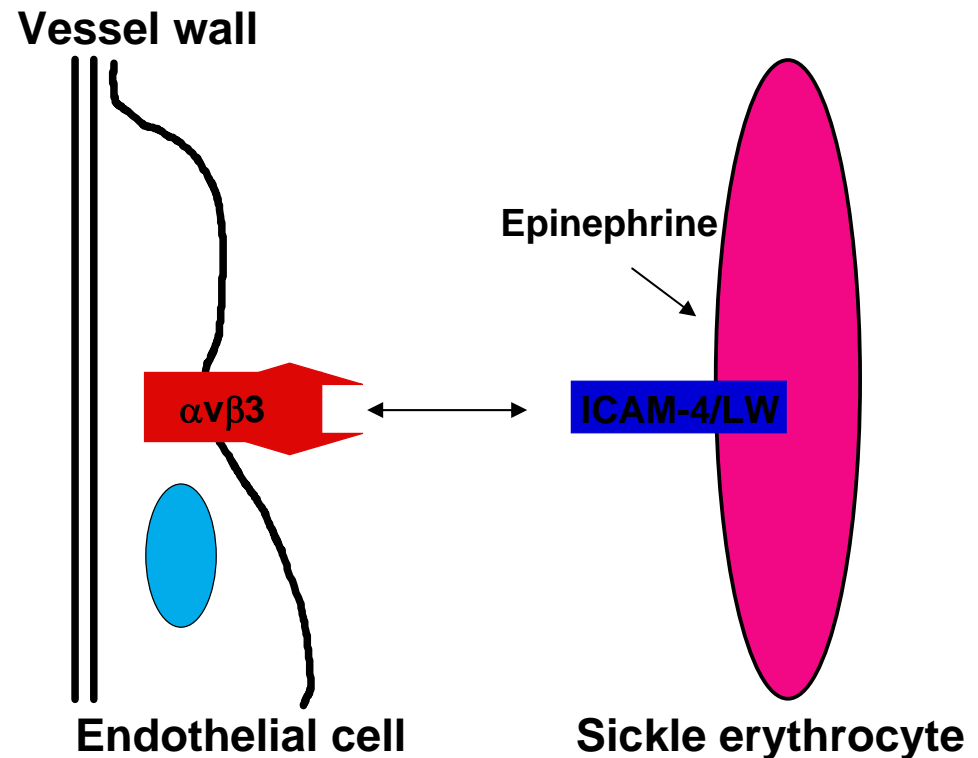
Adhesive interactions between sickle erythrocytes and the endothelium, subendothelial matrix, and plasma ligands



Lancet, 364,1343,2004.

Adhesive Interactions between $\alpha v \beta 3$ and ICAM-4 in Sickle Cell Disease

- Dr. Telen's group showed that epinephrine acts through erythroid signaling pathways (cAMP-dependent protein kinase A) to activate sickle erythrocyte adhesion to endothelium via **ICAM-4 (LW, CD242)- $\alpha v \beta 3$** interactions (Blood, 104,3774,2004).



Integrin $\alpha I I b \beta 3 / \alpha v \beta 3$ aptamer clone 17.16

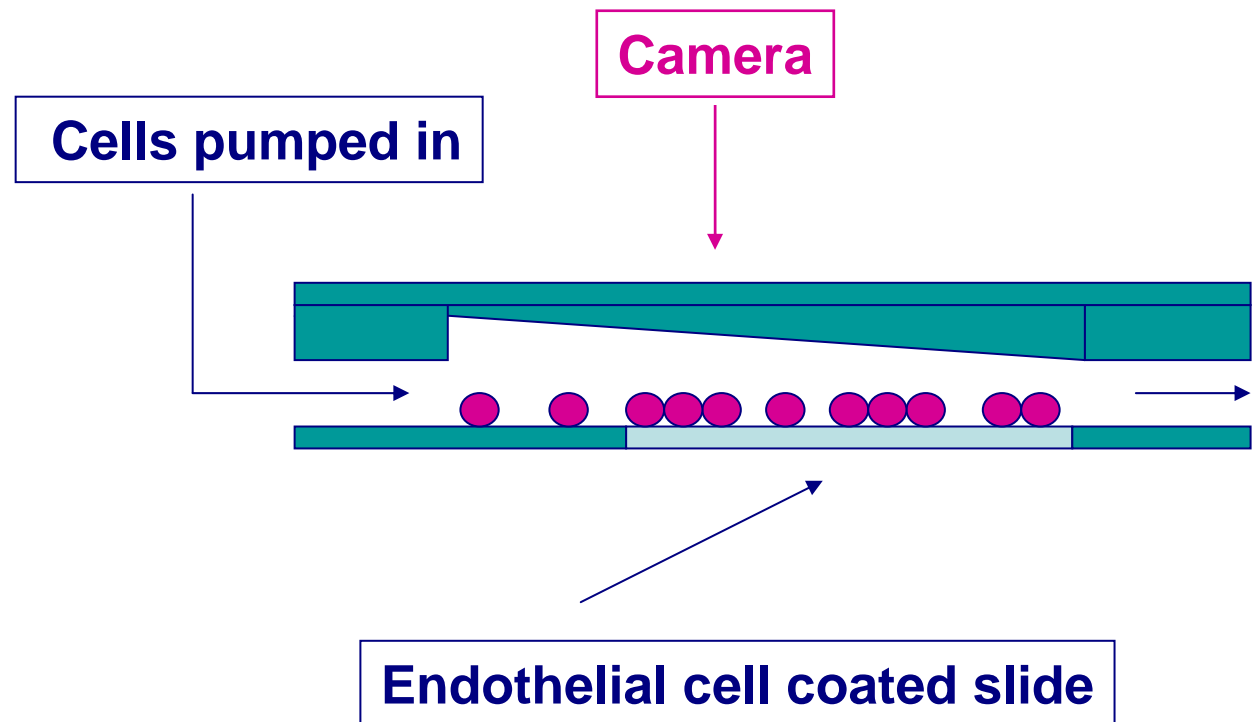
(UUCAACGCUGUGAAGGGCUUAUACGAGCGGAUUACCC);

inhibits $\alpha I I b \beta 3 / \alpha v \beta 3$ binding to its ligand vitronectin/fibrinogen

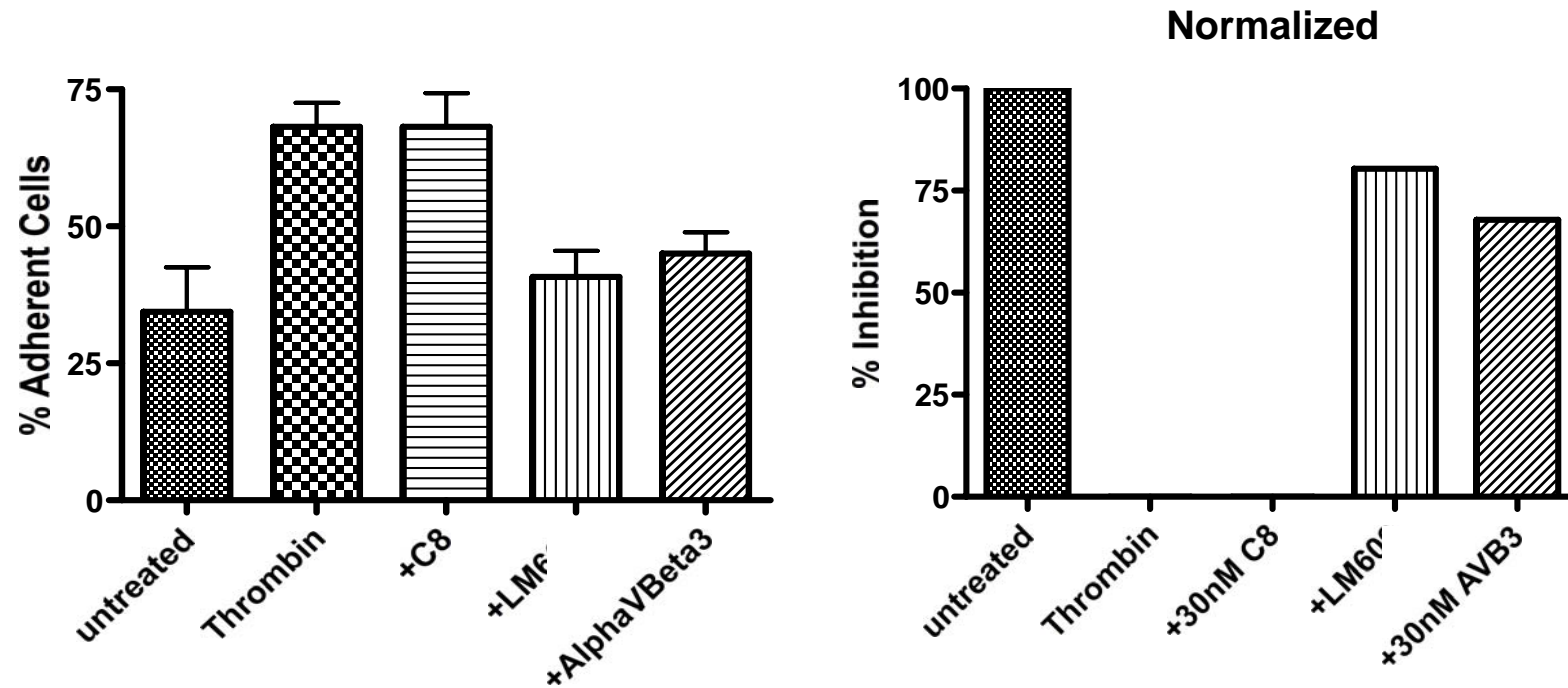
(J Ruckman, United States Patent, 6331394)

Measuring RBC-Endothelial Interactions in a Flow Chamber

- Mimics blood flow through the post capillary venules.
- Maintains physiologic flow rate, shear stress & temperature.
- Glass slides coated with HUVECs (pretreated with thrombin).
- Fluorescently labelled SS RBCs are infused into the chamber.
- Flow is stopped to allow SS RBCs to interact with HUVEC for 15min .
- Flow is then resumed for 10min.



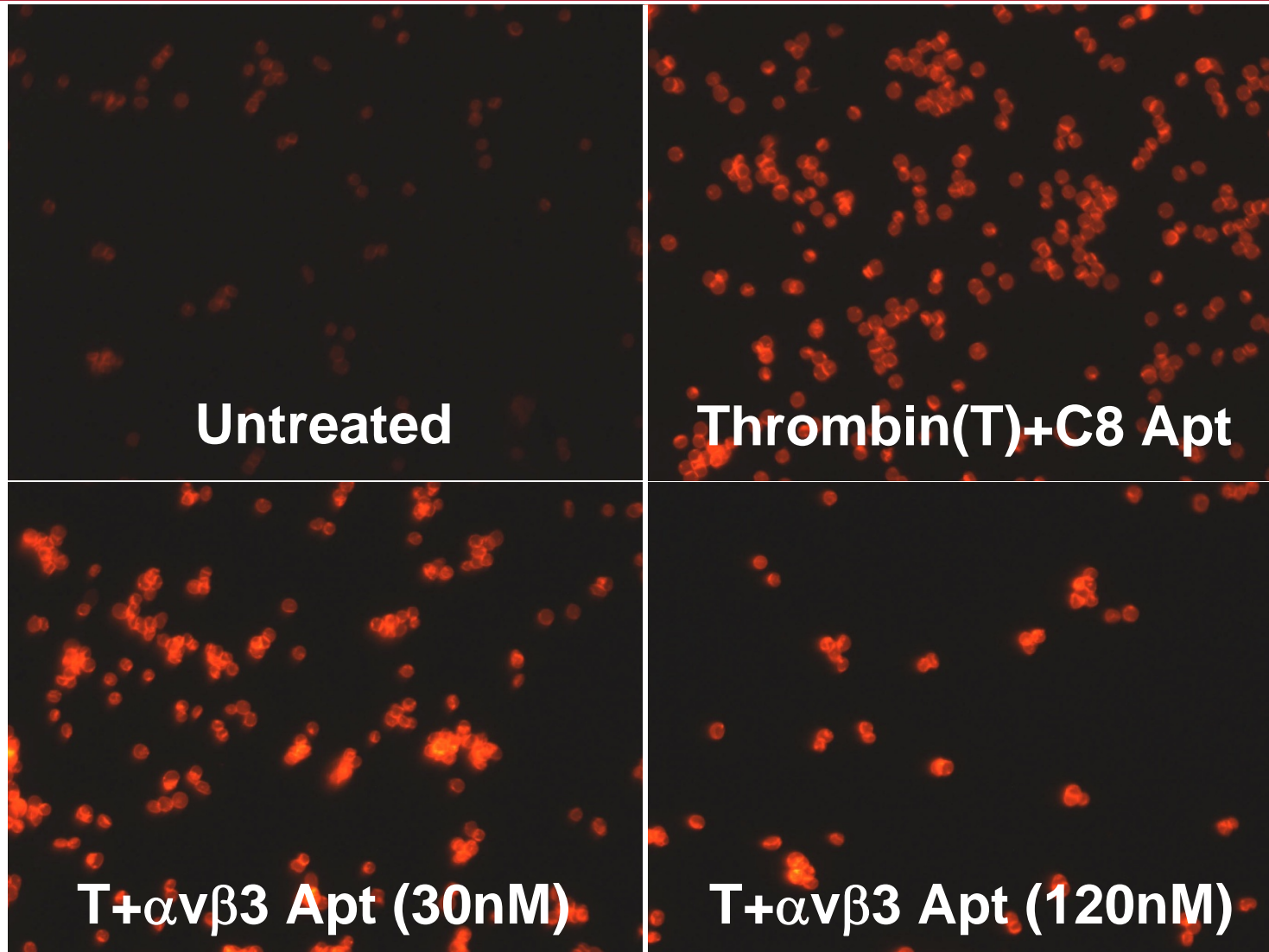
Anti-adhesion Activity of $\alpha v \beta 3$ Binding Aptamer Clone (17.16)



Aptamer clone 17.16 (30nM) had anti-adhesion activity similar to LM609 (an antibody that inhibits SS RBC adhesion to $\alpha v \beta 3$), whereas human complement 8 aptamer did not have anti-adhesion activity.

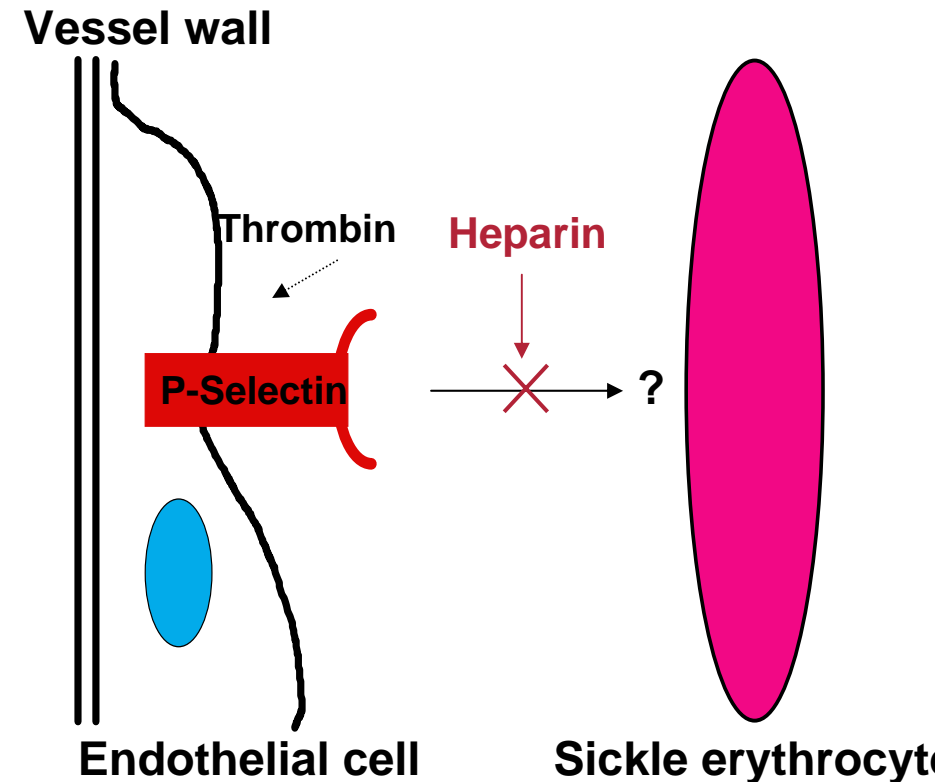
Normalized % inhibition of aptamer clone 17.16 (30nM) at 2 dynes/cm² was 68%.

Representative Pictures of Enhanced Anti-adhesion Activity of $\alpha v \beta 3$ Aptamer Clone (17.16)



P-Selectin Adhesive Interactions in Sickle Cell Disease

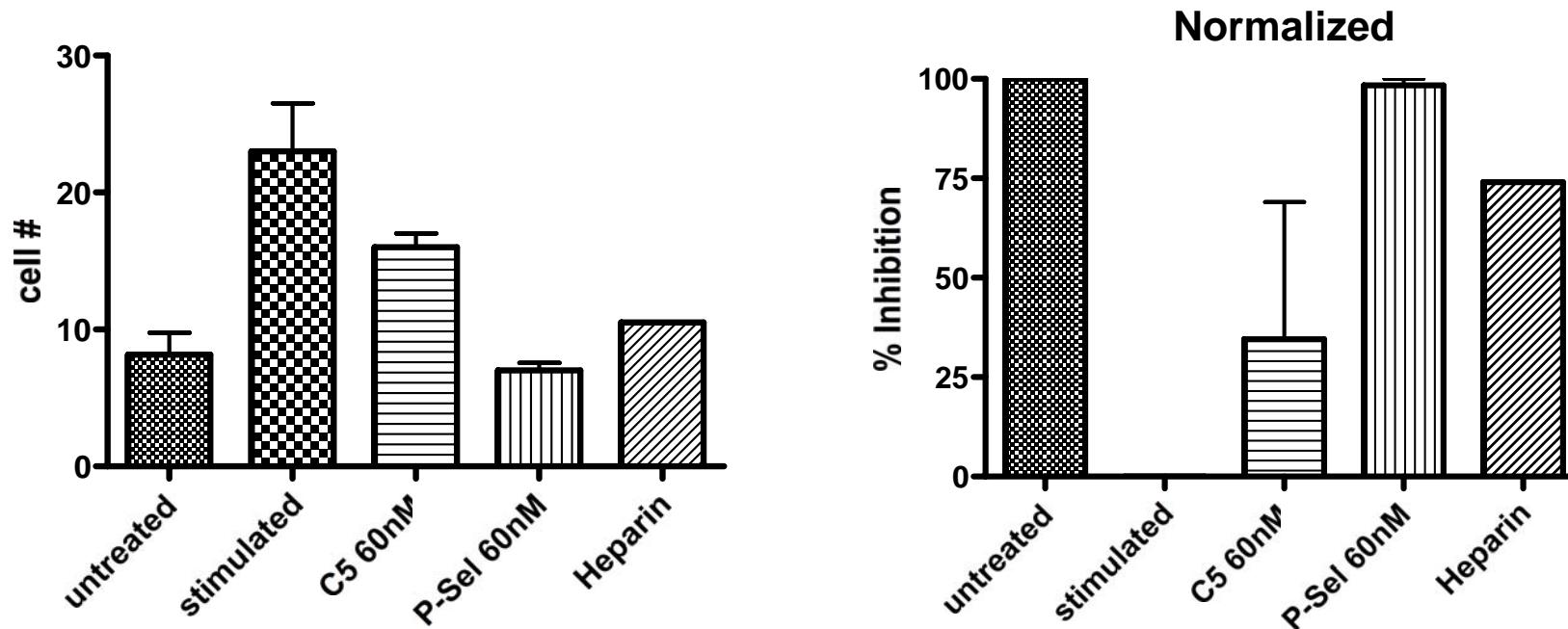
- The endothelial expression of **P-Selectin (CD62P)** treated with thrombin mediates the adhesion of sickle erythrocytes to the endothelium (Blood, 98,1955,2001).
- **Heparin** inhibits the adhesion of sickle erythrocytes to P-selectin (Blood, 100,3790,2002).



P-Selectin aptamer clone PF377

(ACGCUCAACGAGCCAGGAACAUCGACGUCAGCAAACGCGAGCGCAACCAGUAACACC);
inhibit P-selectin binding to its ligand sialyl Lewis X, and inhibit neutrophil-platelet adhesion (Jenison RD, Antisense Nucleic Acid Drug Dev, 8,265,1998)

Anti-adhesion Activity of P-Selectin Binding Aptamer Clone (PF377)



Aptamer clone PF377 (60nM) had anti-adhesion activity greater than heparin (inhibitor of SS RBC adhesion to P-Selectin). Normalized % inhibition of aptamer clone PF377 (60nM) at 1 dynes/cm² was 98%.

In Vivo Flow Chamber Assays

- Fluorescein-labeled RBC is injected intravenously into the tail of female nude mice in which window chambers have been placed.
- Intravital microscopy and video recording are performed.



Normal RBC



Activated SS-RBC

Summary

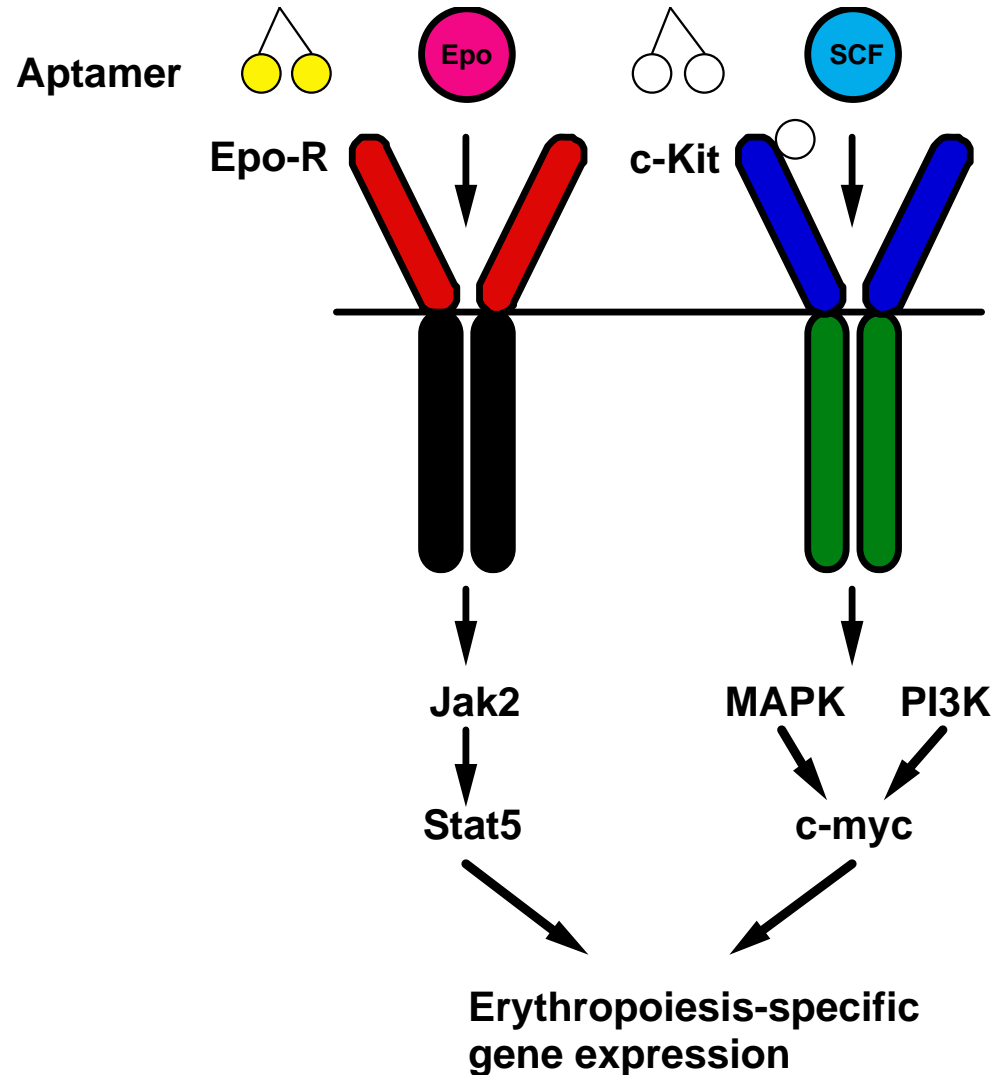
- **Integrin $\alpha v \beta 3$ aptamer clone (17.16) and P-Selectin aptamer clone (PF377) had strong bindings and anti-adhesion activities.**
- **These aptamer clones are currently undergoing truncation experiments and 2'OMe substitutions to enhance stability for in vivo applications.**
- **In vivo studies are currently preparing to test these aptamer clones.**

Conclusions

- Aptamers may be superior to antibodies for certain therapies.
- Aptamers can be modified to have greatly enhanced plasma stability and longer circulating half-lives.
- Experience to date suggests that aptamers have no~low toxicity and immunogenicity in vivo.
- “Antidotes”, which are short complementary sequences (antisense) to the aptamers, can reverse the properties of aptamers. Aptamer-antidote pairs are safe and highly regulatable drugs.
- Aptamer can mediate siRNA delivery; Cell type-specific delivery of siRNA with aptamer-siRNA chimeras.
- Aptamers can be isolated by a simple in vitro process for virtually **any target**, even those that are toxic or have low immunogenicity, if **purified target and its screening assay system** are available.

Future Directions

Anemia and beyond: new frontiers of erythropoietin research



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