ISOFORME VEGF e MACULA

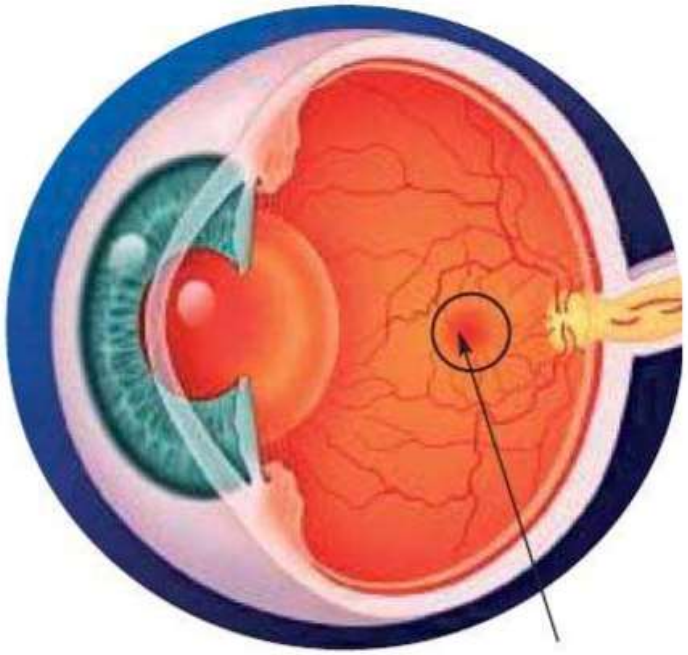
VEGF and Macula Degeneration

- Both clinical and biological findings have implicated **vascular endothelial growth factor (VEGF)** in the pathophysiology macular edema and degeneration.
- ***VEGF** is both a potent enhancer of vascular permeability and a key inducer of angiogenesis.
- *

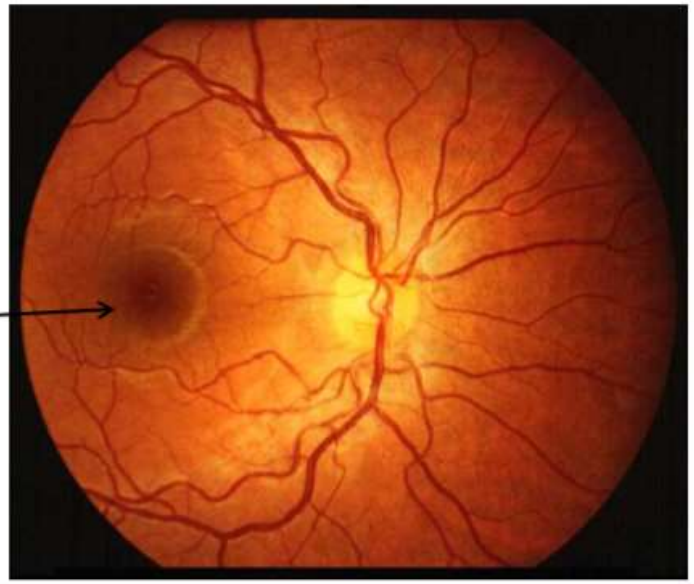
VEGF and Macula Degeneration 2

- ***VEGF** levels are elevated in the eyes of patients.
- Injection of **VEGF (the VEGF165 isoform in particular)** into healthy eyes of animals can induce associated ocular pathologies

La maculopatia senile umida è causata dalla crescita di vasi sanguigni anomali, che danneggiano l'area dell'occhio responsabile della visione centrale, che è essenziale per la maggior parte delle attività visive



la macula

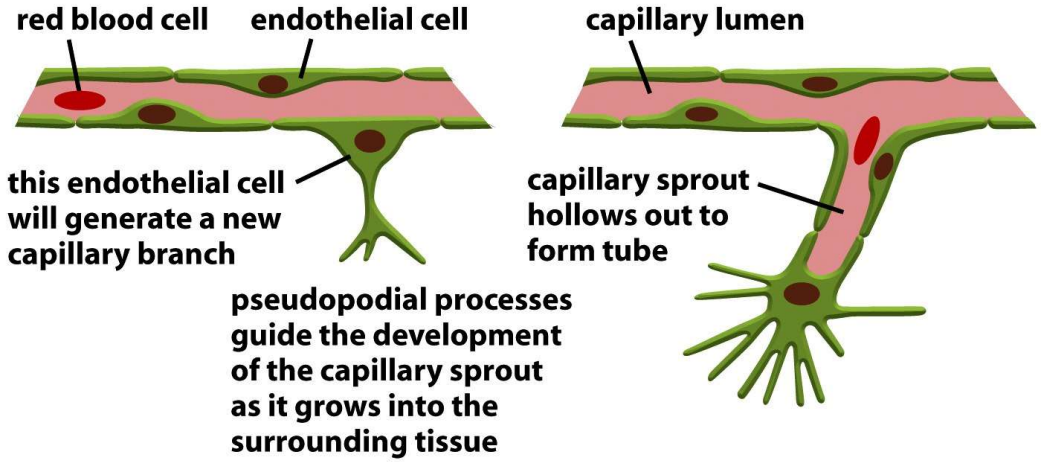


EDEMA MACULARE DIABETICO

- ✓ aumento di spessore della retina centrale
- ✓ presenza di essudati

Angiogenesis:

Sprouting of cells from mature endothelial cells of the vessel wall



(secretion of proteases, resolution of Basal lamina, migration towards Chemotactic gradient, proliferation, Tube formation)

VEGF is factor largely specific for endothelial cells,

bFGF can also induce, not specific for EC)

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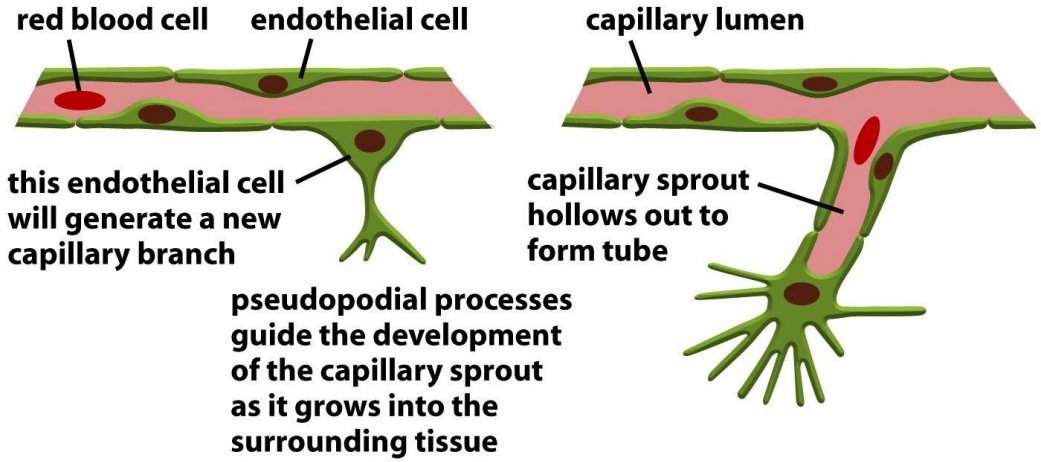

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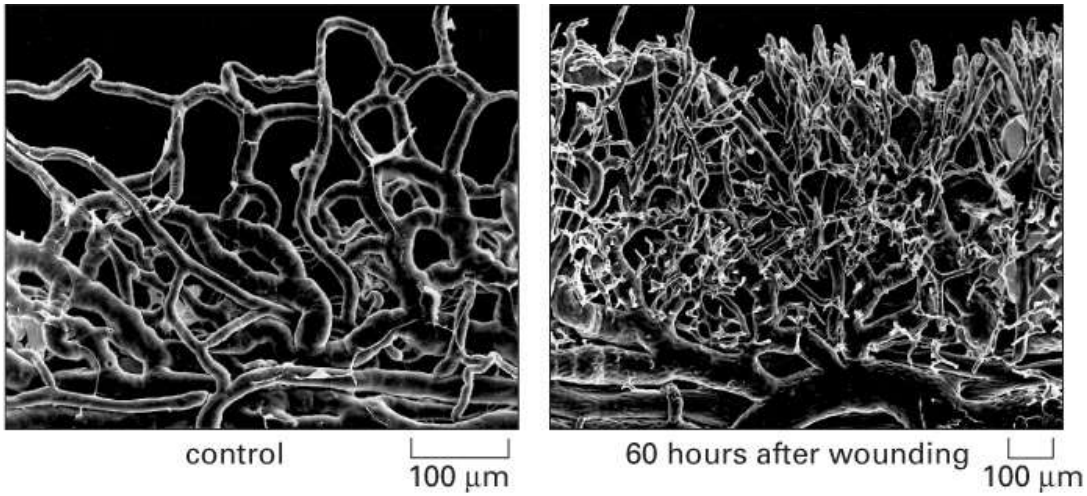
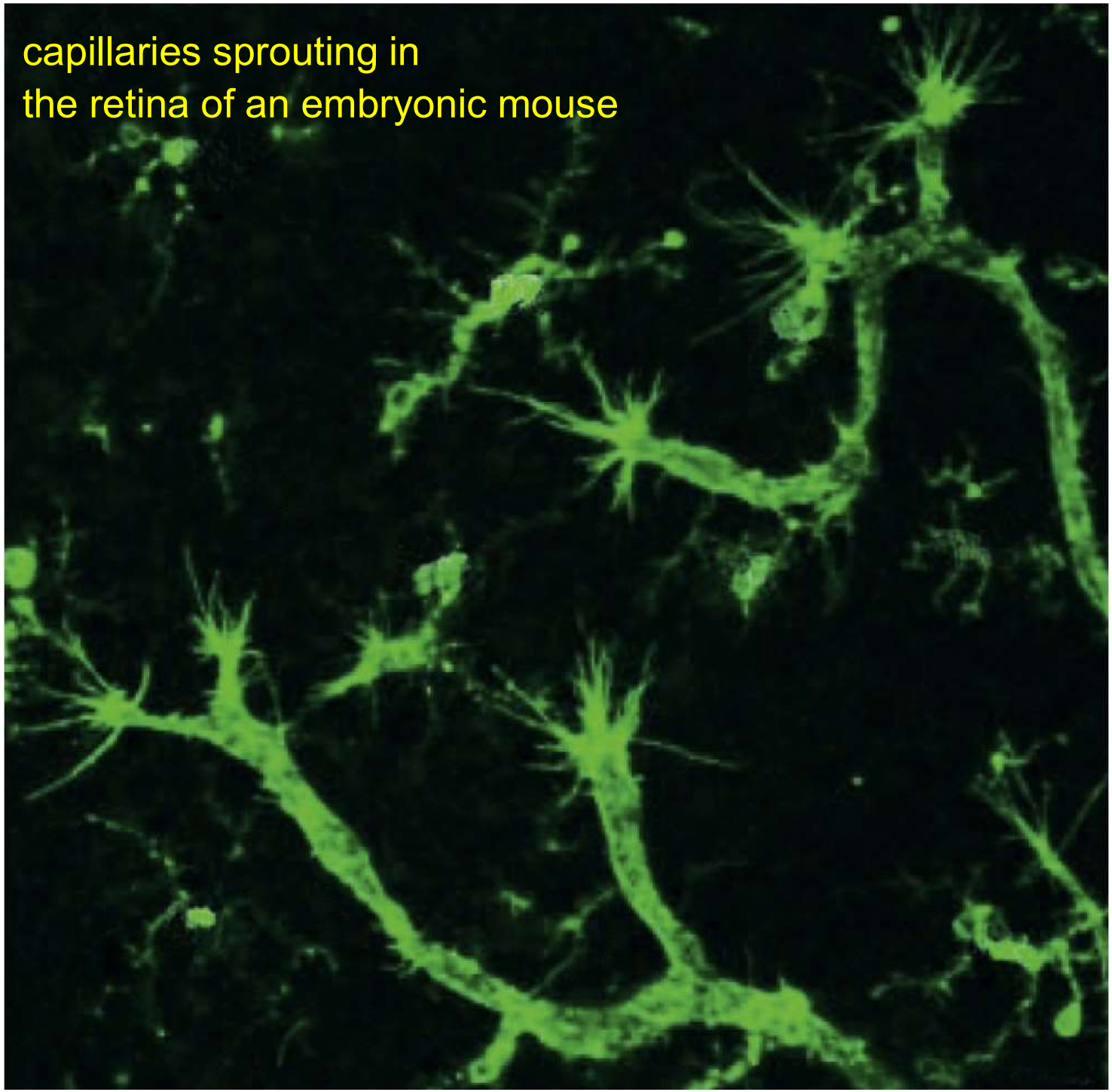


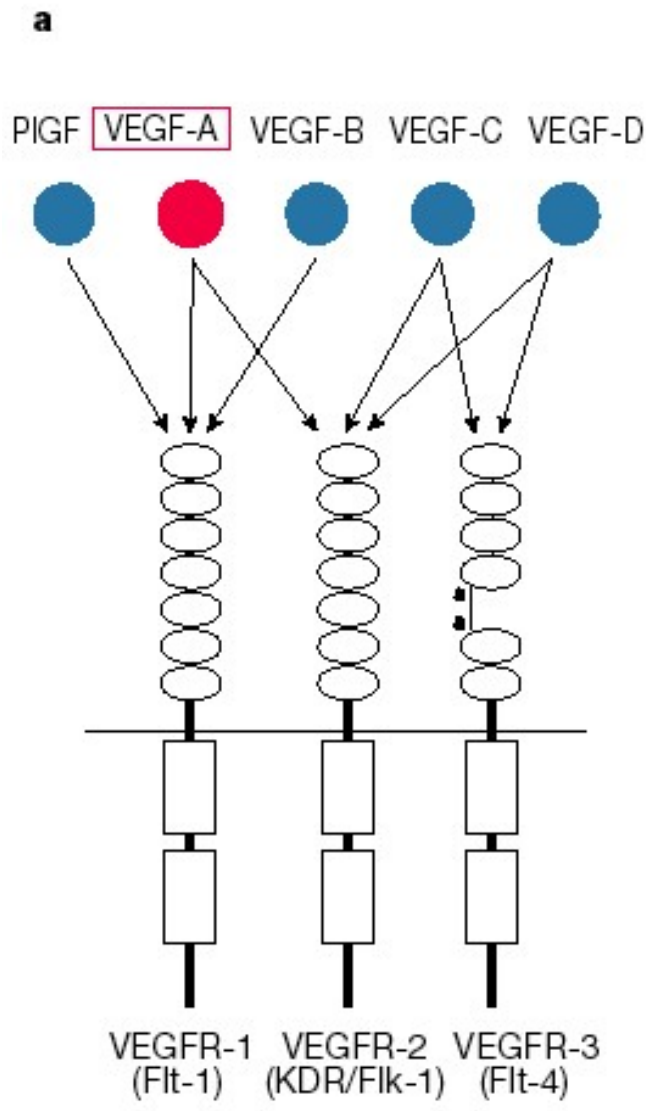
Figure 22–27. Molecular Biology of the Cell, 4th Edition.

Mouse cornea: wounding induces angiogenesis, chemotactic response to angiogenic factors

capillaries sprouting in
the retina of an embryonic mouse



VEGF/VEGFR family

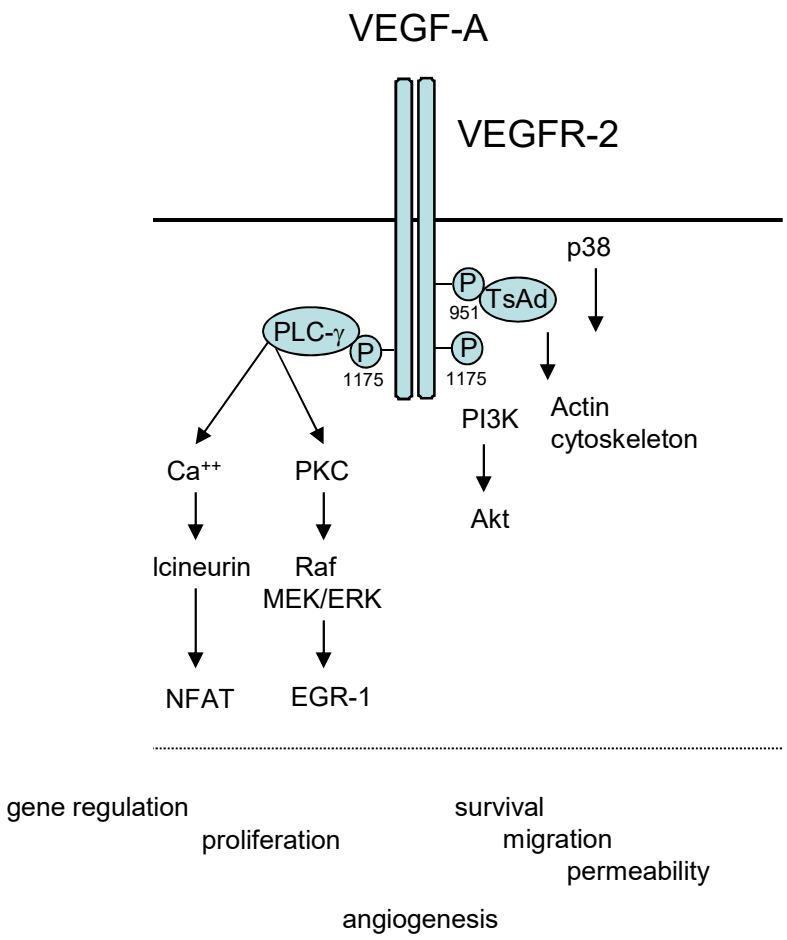


VEGF/VEGFR:
VEGF-A: initiation of vasculogenesis and sprouting angiogenesis,
Immature vessels,
Vascular permeability factor,
Haploid insufficiency in k.o. mice,

PlGF: remodeling of adult vessels
VEGF-B: heart vascularization ?
VEGF-C: lymphatic vessels
VEGF-D: lymphatic vessels ?

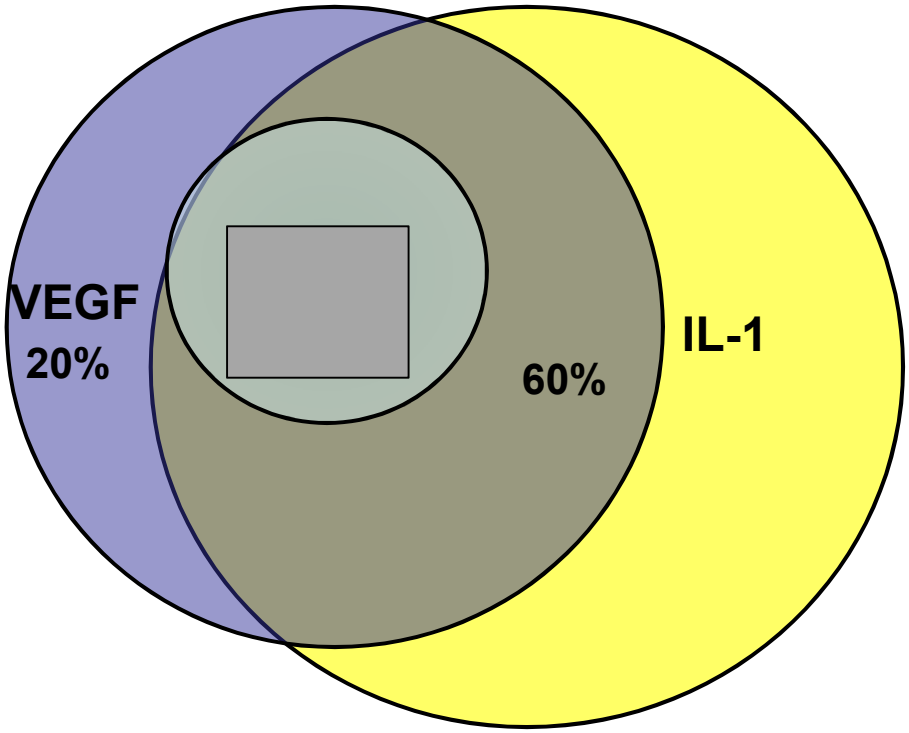
VEGFR-2: growth and permeability
VEGFR-1: negative role ? decoy receptor
VEGFR-3: lymphatic vessels

Signaling by receptors of endothelial cells



Hofer E., Schweighofer B. Signaling transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis ang haemostasis 2007

Overlapping and specific gene repertoires of VEGF and IL-1

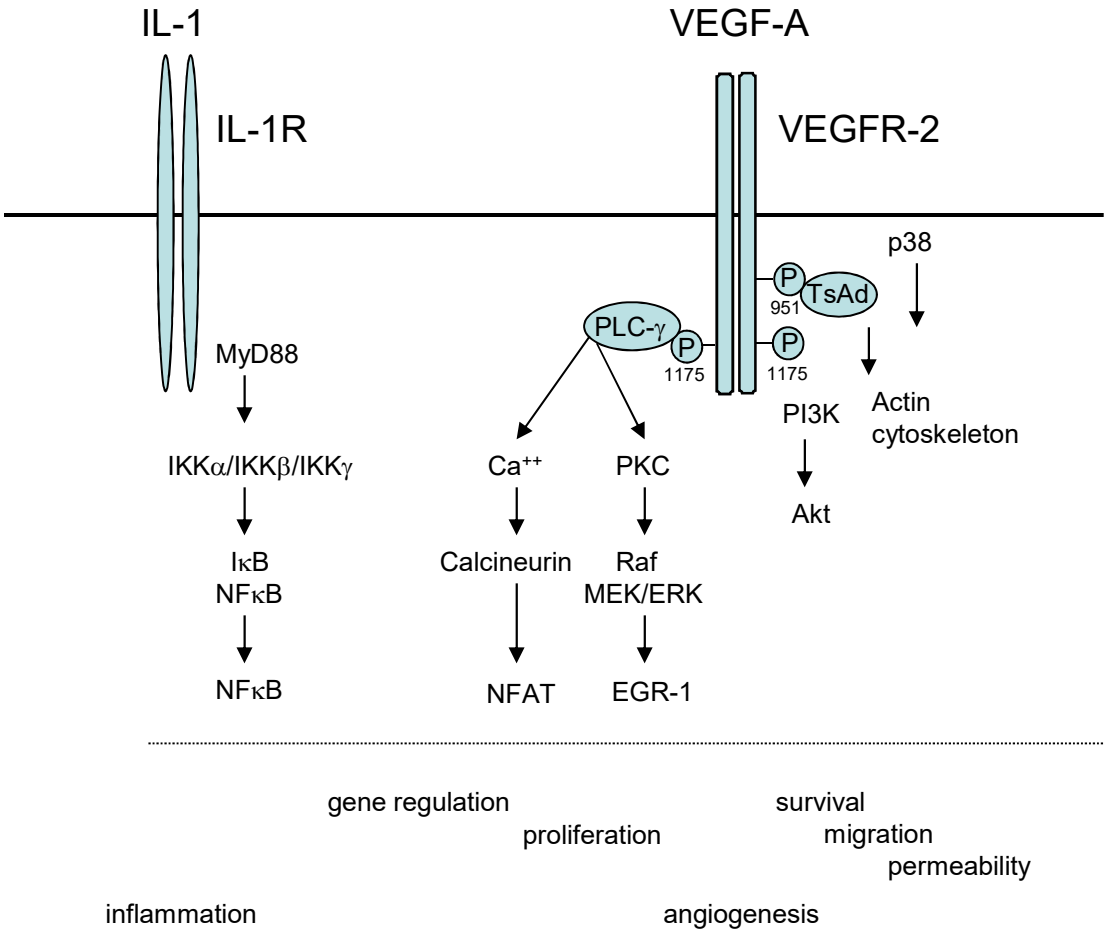


About 60 genes reproducibly induced by VEGF over 3-fold

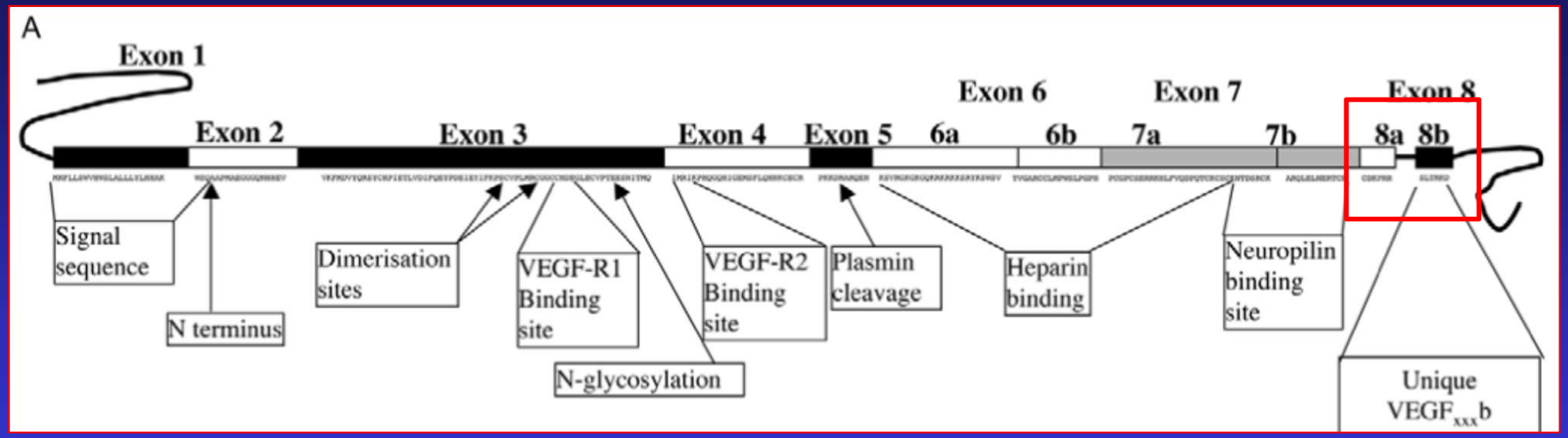
VEGF-induced genes overlap to a large degree with IL1-induced genes (50-60 %)

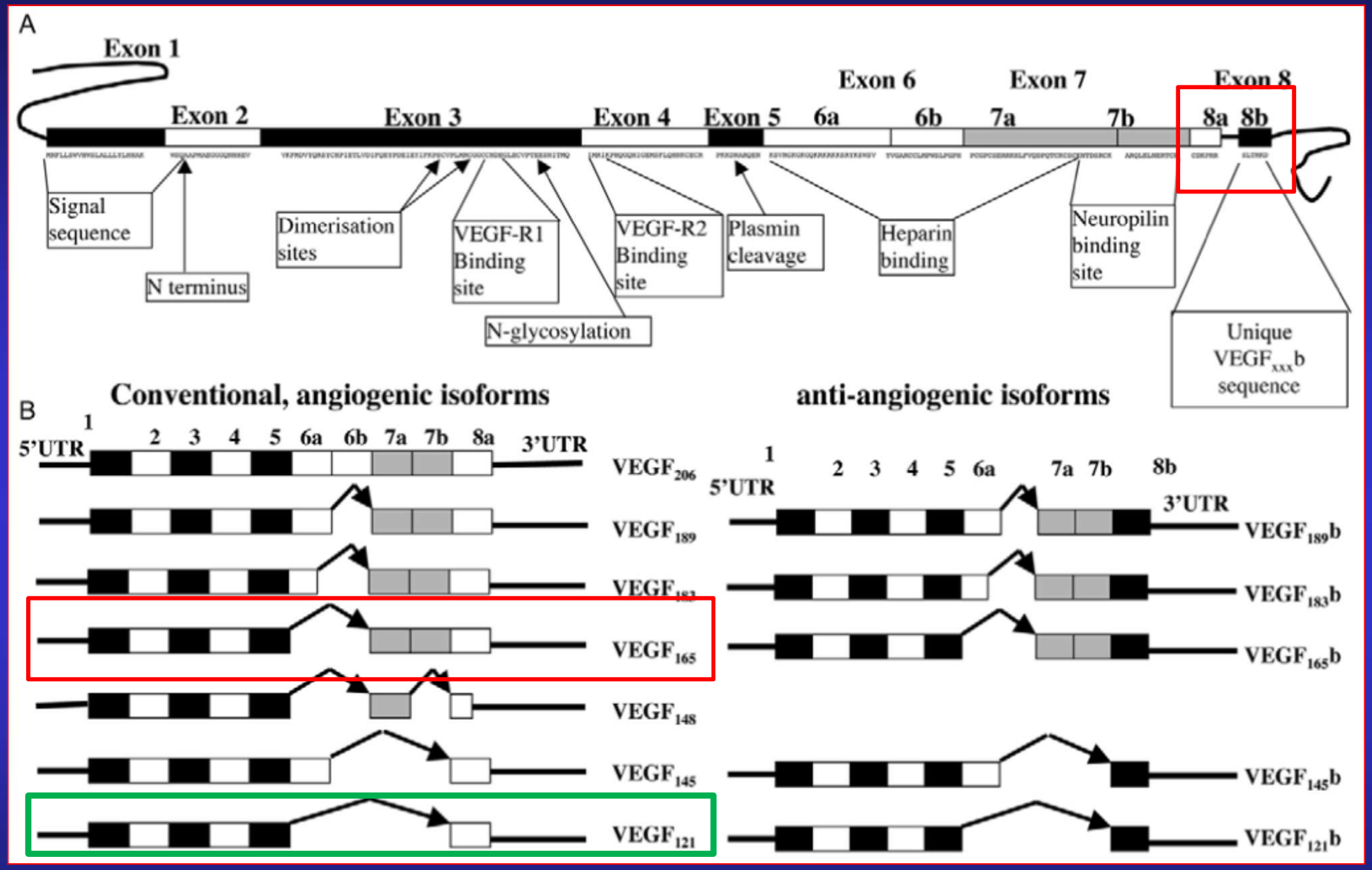
20 % of genes are preferentially induced by VEGF

Signaling by receptors of endothelial cells



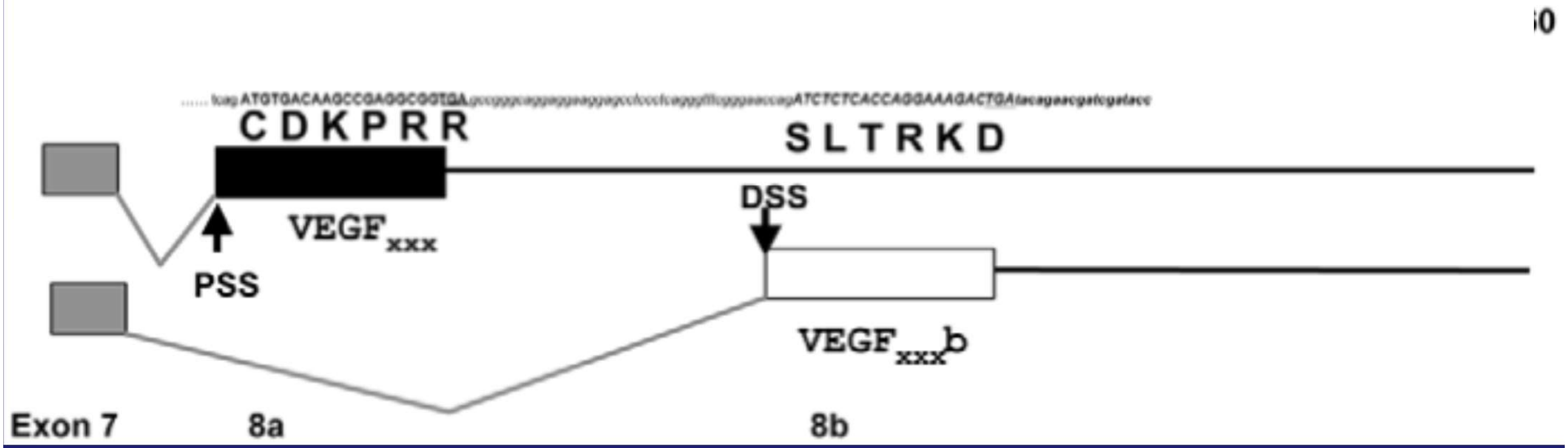
Hofer E., Schweighofer B. Signaling transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis ang haemostasis 2007

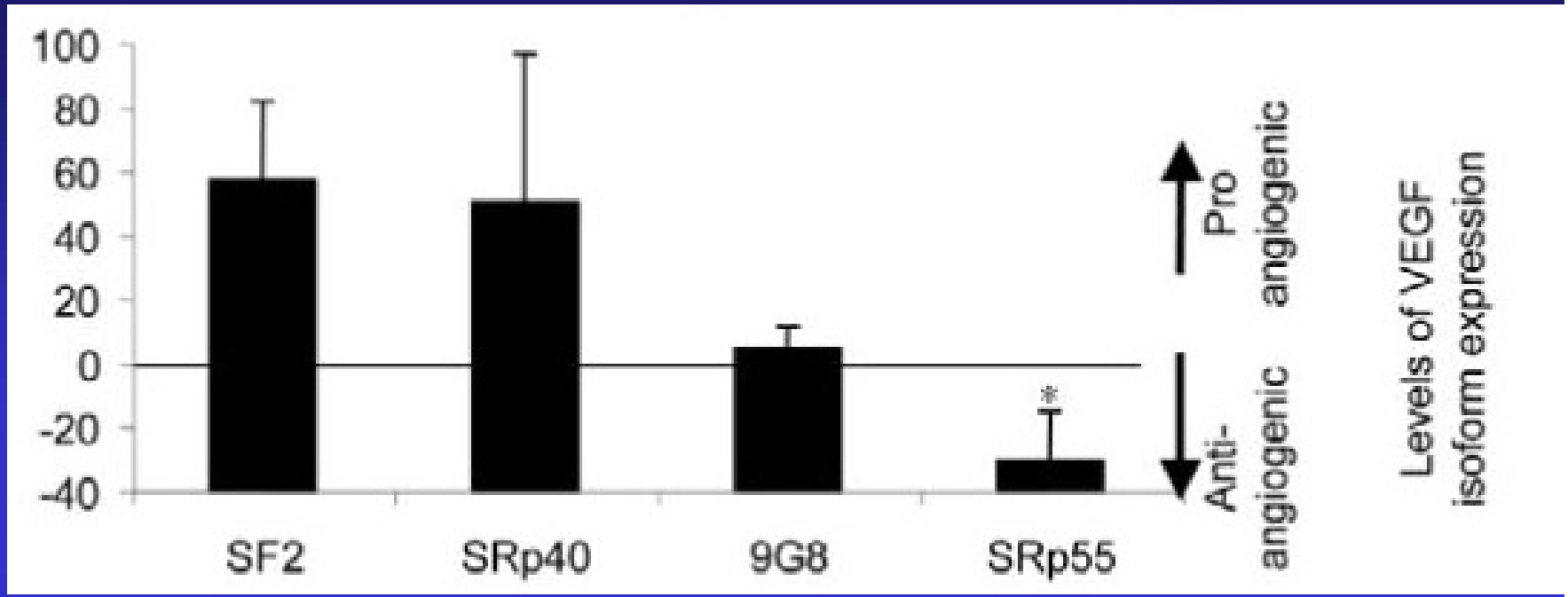

A
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
6a
6b
Exon 7
7a
7b
Exon 8
8a
8b
Signal sequence
N terminus
Dimerisation sites
VEGF-R1 Binding site
N-glycosylation
VEGF-R2 Binding site
Plasmin cleavage
Heparin binding
Neuropilin binding site
Unique VEGF$_{xxx}$b


A
Exon 1
Exon 2
Exon 3
Exon 4
Exon 5
Exon 6
6a
6b
Exon 7
7a
7b
Exon 8
8a
8b
Signal sequence
N terminus
Dimerisation sites
VEGF-R1 Binding site
N-glycosylation
VEGF-R2 Binding site
Plasmin cleavage
Heparin binding
Neuropilin binding site
Unique VEGFxxxb sequence
B
Conventional, angiogenic isoforms
anti-angiogenic isoforms
5'UTR
1
2
3
4
5
6a
6b
7a
7b
8a
8b
3'UTR
VEGF206
VEGF189
VEGF183
VEGF165
VEGF148
VEGF145
VEGF121
VEGF189b
VEGF183b
VEGF165b
VEGF145b
VEGF121b

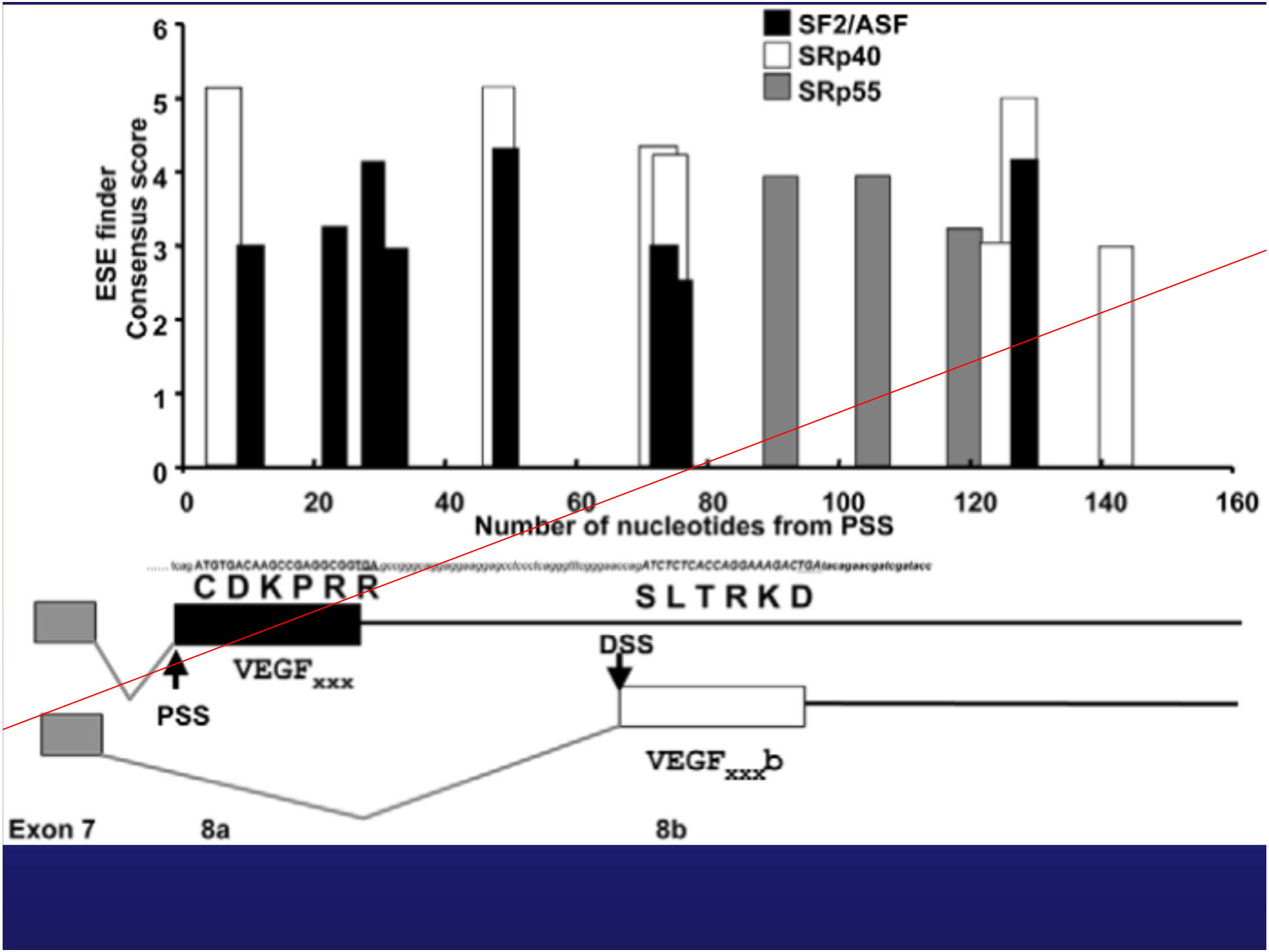
Proximal splice-site selection (PSS)

Distal splice-site selection DSS





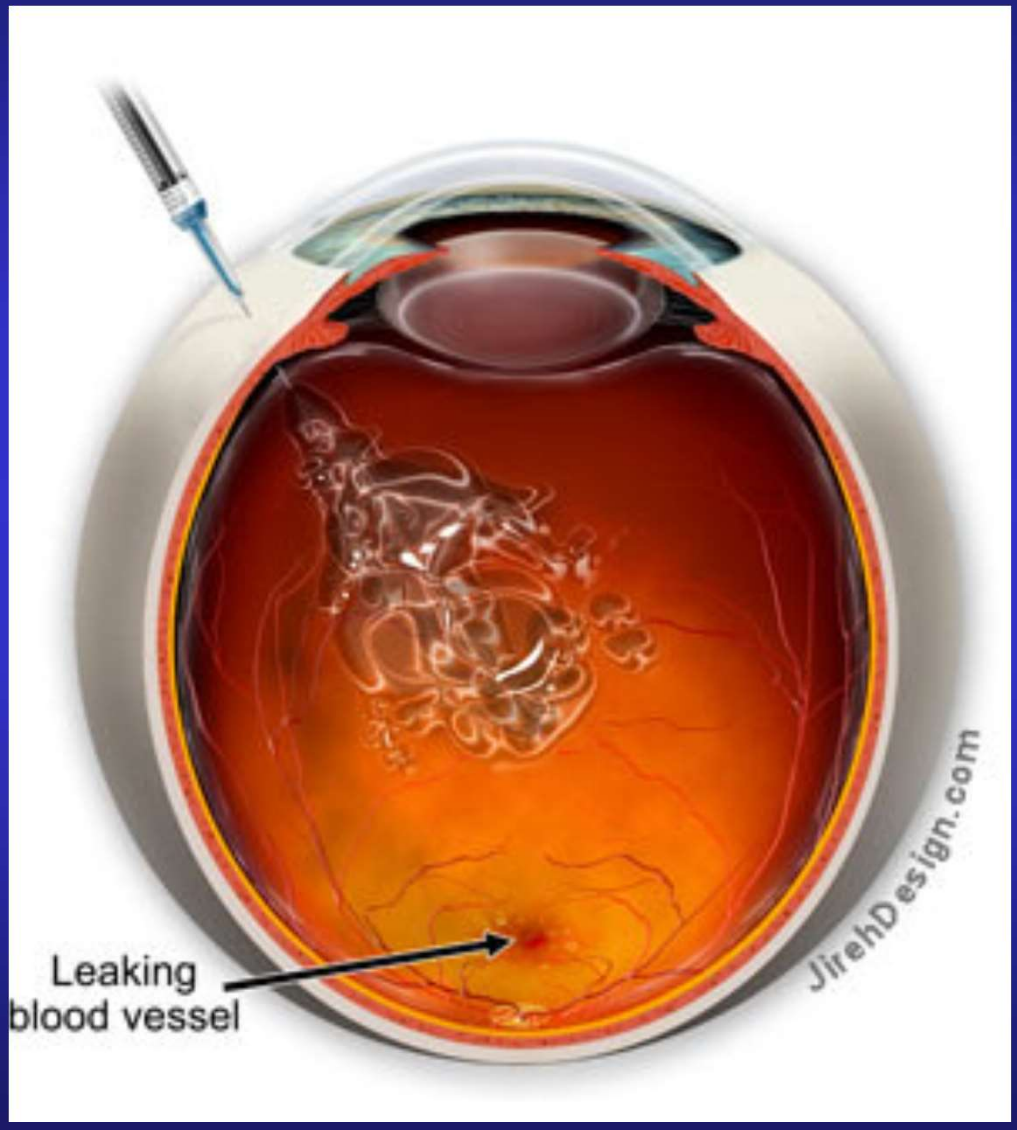
Effect of overexpression of splicing factors on VEGF isoform production.


SF2/ASF
SRp40
SRp55
ESE finder
Consensus score
0
1
2
3
4
5
6
0
20
40
60
80
100
120
140
160
Number of nucleotides from PSS
C D K P R R
S L T R K D
DSS
VEGF xxx
PSS
VEGF xxx b
Exon 7
8a
8b

Vascular Endothelial Growth Factor and the Potential Therapeutic Use of Pegaptanib (Macugen®) in Diabetic Retinopathy

Starita C, Patel M, Katz B, Adamis A

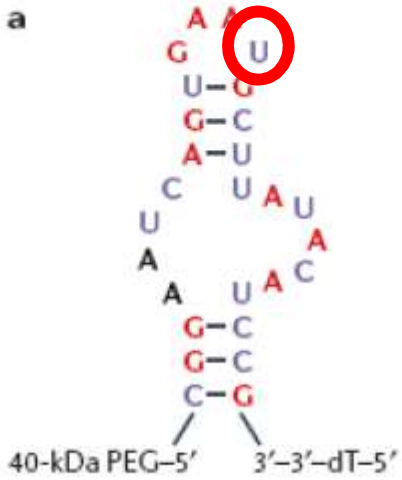
- Pegaptanib, an RNA aptamer used in the treatment of age related macular degeneration, binds and inactivates VEGF165.
- In animal models it reverses the blood-retinal barrier breakdown.


Leaking
blood vessel
JirehDesign.com

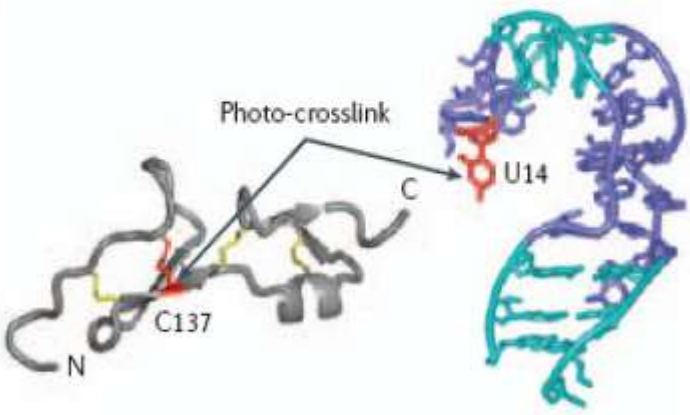
Il pegaptanib e` un antagonista selettivo del $VEGF_{165}$

Il Pegaptanib e` un aptamero a filamento singolo di **RNA** formato da 28 nucleotidi legato a 2 molecole di 20-kDa di glicole polietilenico (PEG)

E` dotato di alta affinita` per il $VEGF_{165}$ (**vascolarizzazione patologica**) e nessun legame con il $VEGF_{121}$ (**vascolarizzazione fisiologica**)

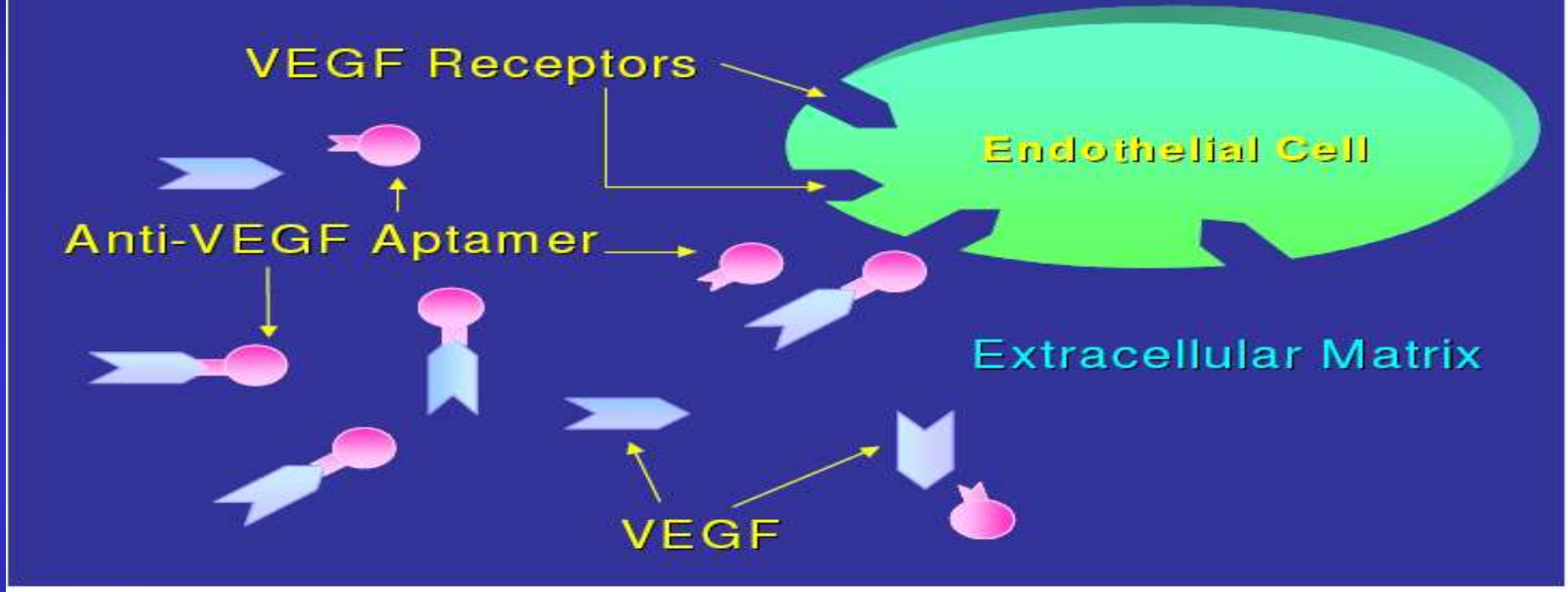


a | Sequenza e struttura secondaria del pegaptanib.



Il legame avviene tra la cisteina – 137 del $VEGF_{165}$ e l`uridina-14 dell`$aptamero_{14}$ (in rosso).

Extracellular Neutralization of VEGF

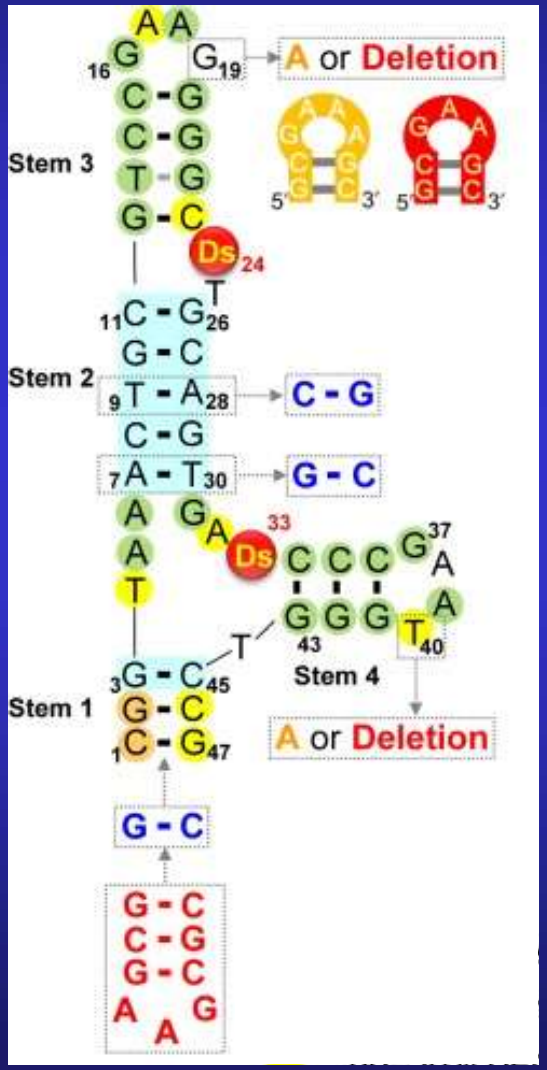


- Il Pegaptanib si lega specificamente al VEGF-165, impedendone l'aggancio con il suo recettore

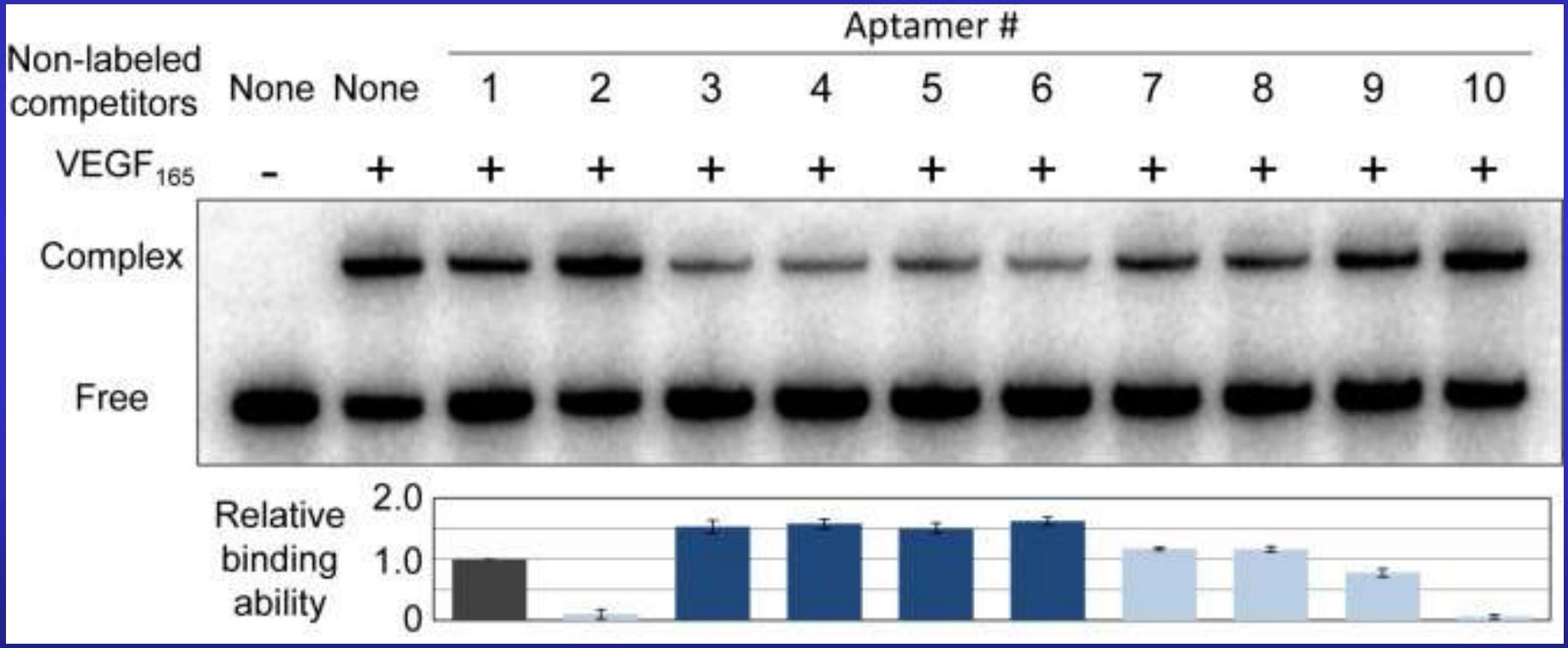
Responders nello studio V.I.S.I.O.N.
Lesioni iniziali vs tutti I Pazienti

* Lesione iniziale definita come: occulta, senza essudati, e occhio controlaterale con visus migliore

DNA anti-VEGF165 DNA aptamers

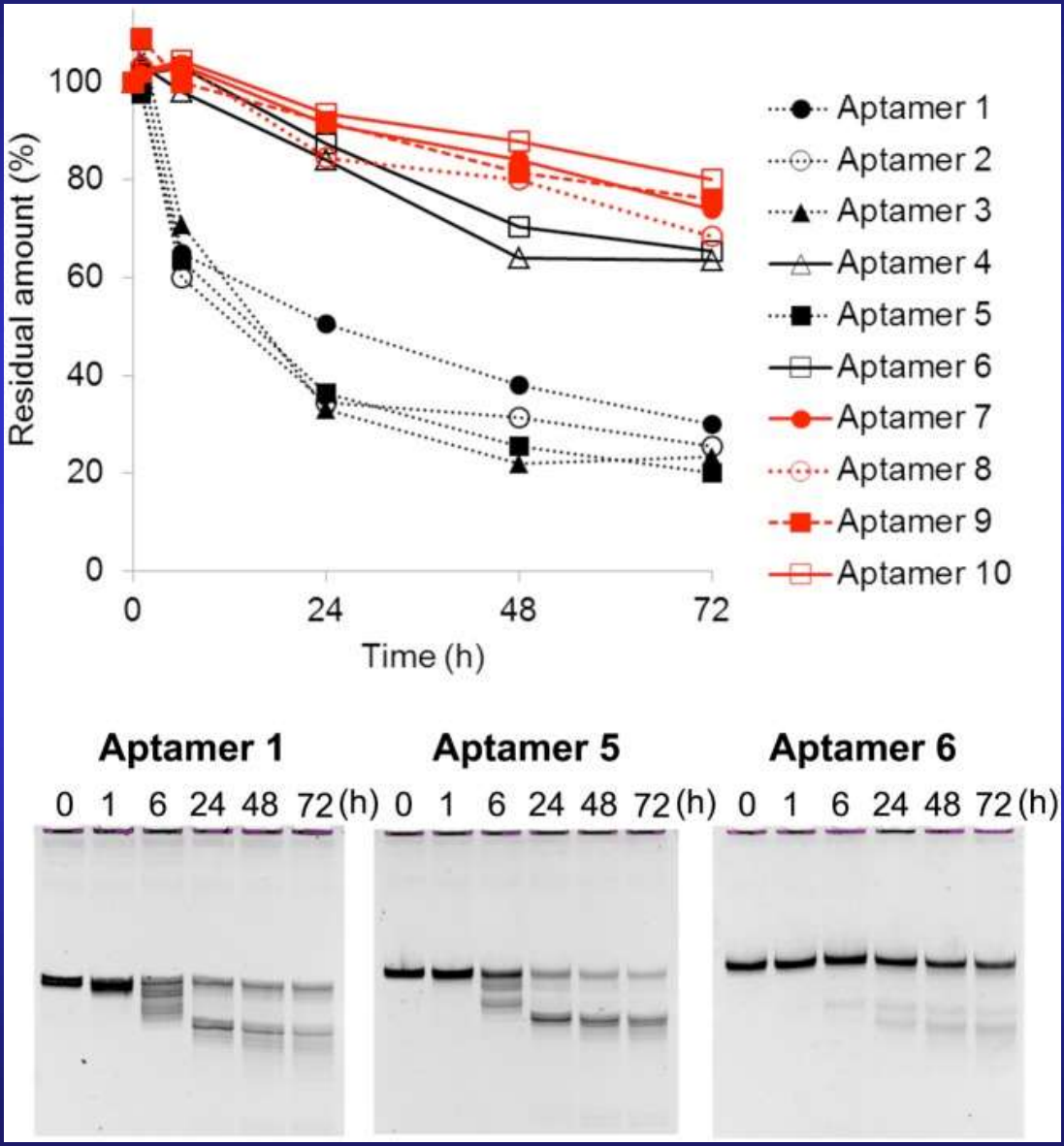


competition assay

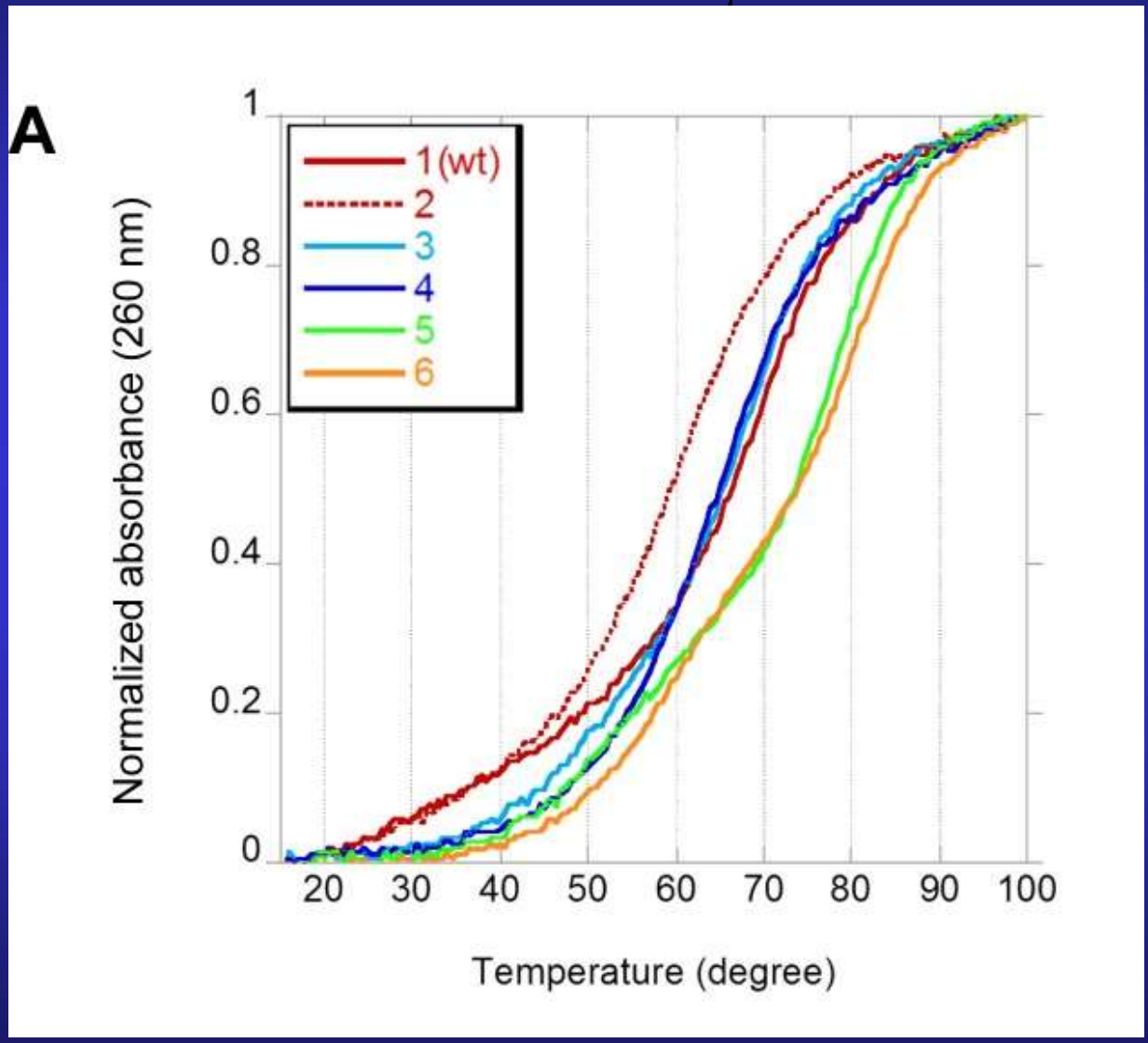


32P-labeled Aptamer 1 (100 nM) was incubated with VEGF165 (100 nM), in the presence of each non-labeled variant as a competitor (100 nM), at 37°C for 30 min

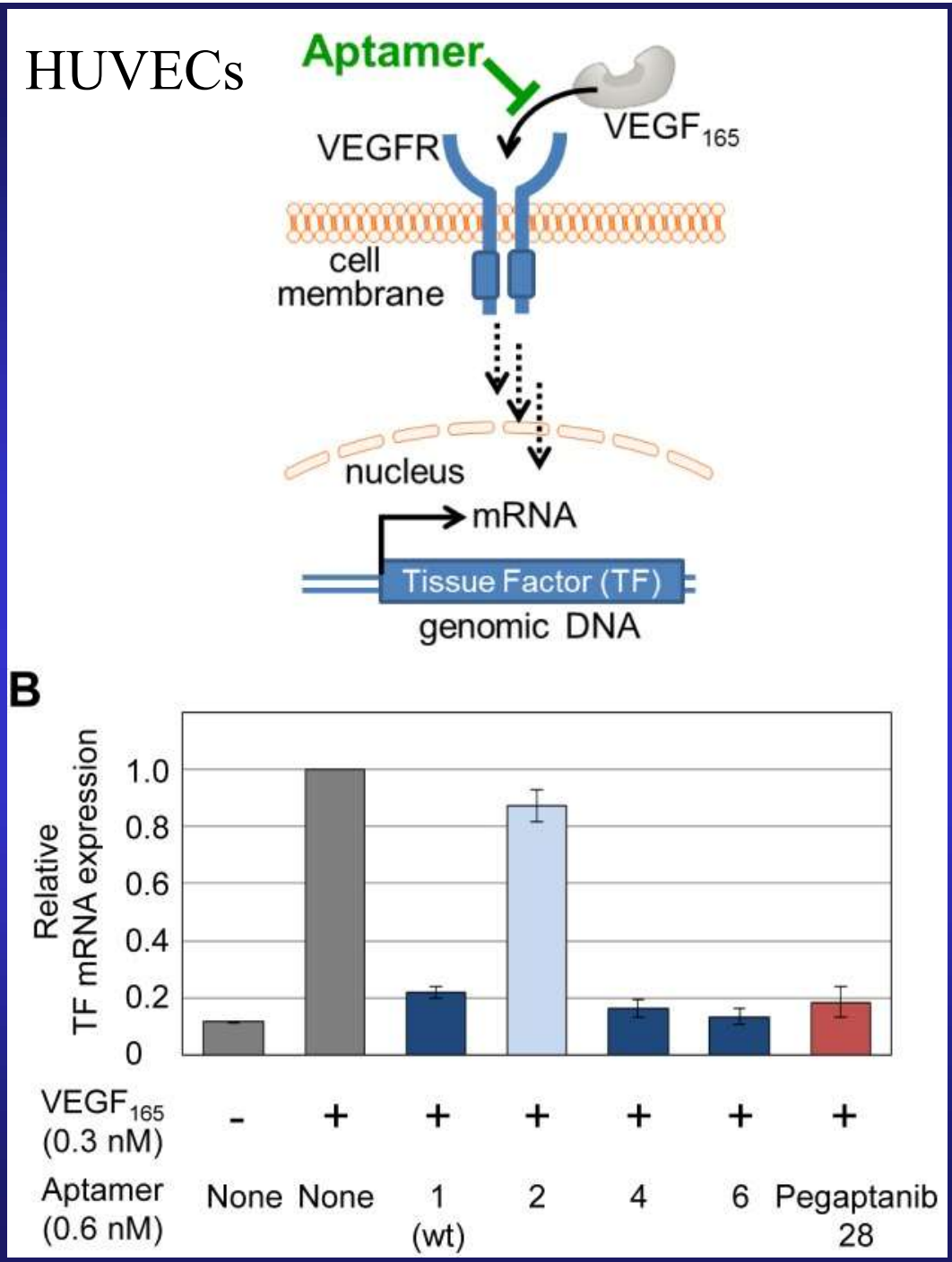
Nuclease resistance of anti-VEGF165 aptamers in human serum



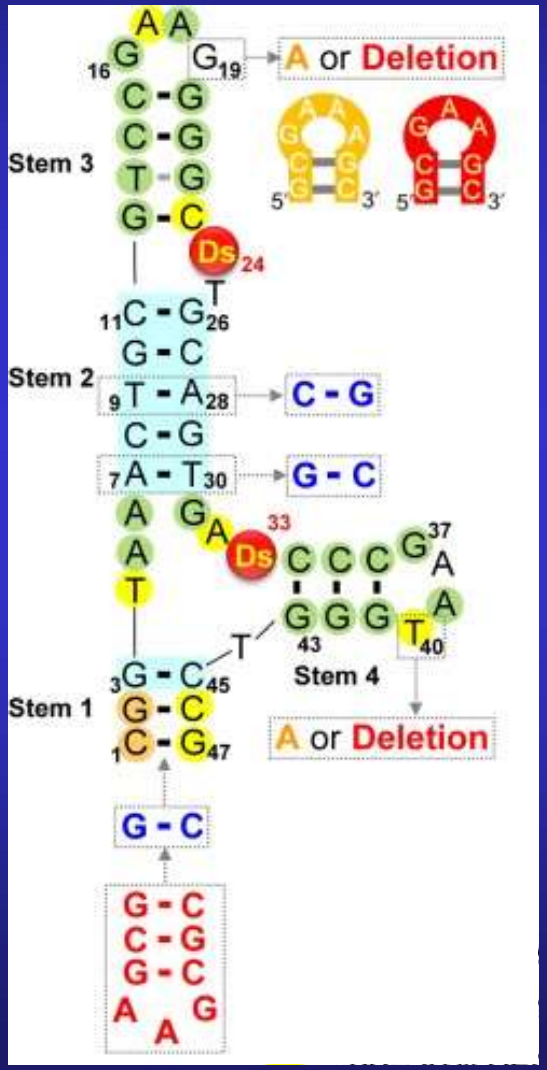
Thermal stabilities of aptamers



Inhibition of the interaction between VEGF165 and its receptor by aptamers

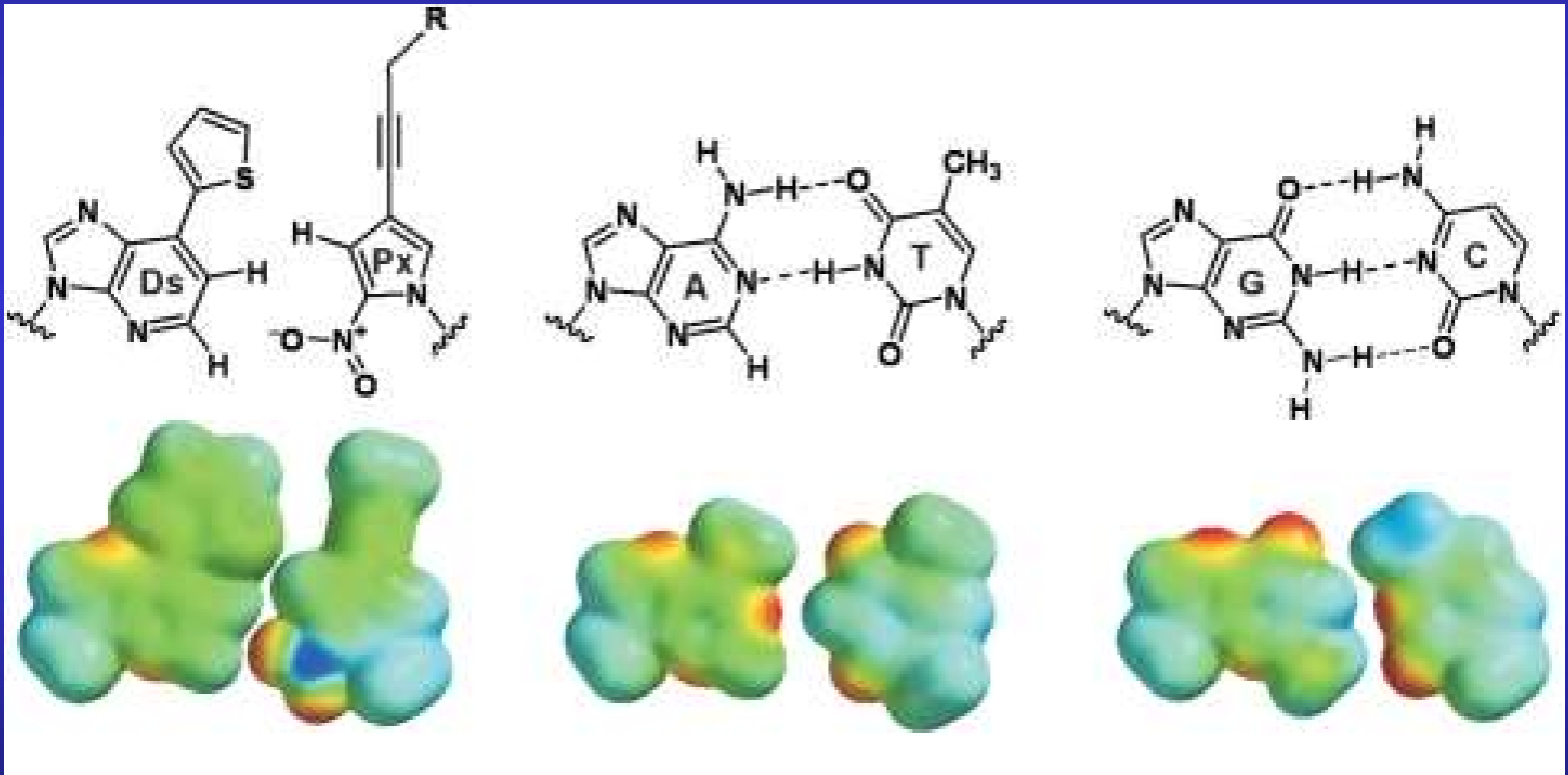


DNA anti-VEGF165 DNA aptamers

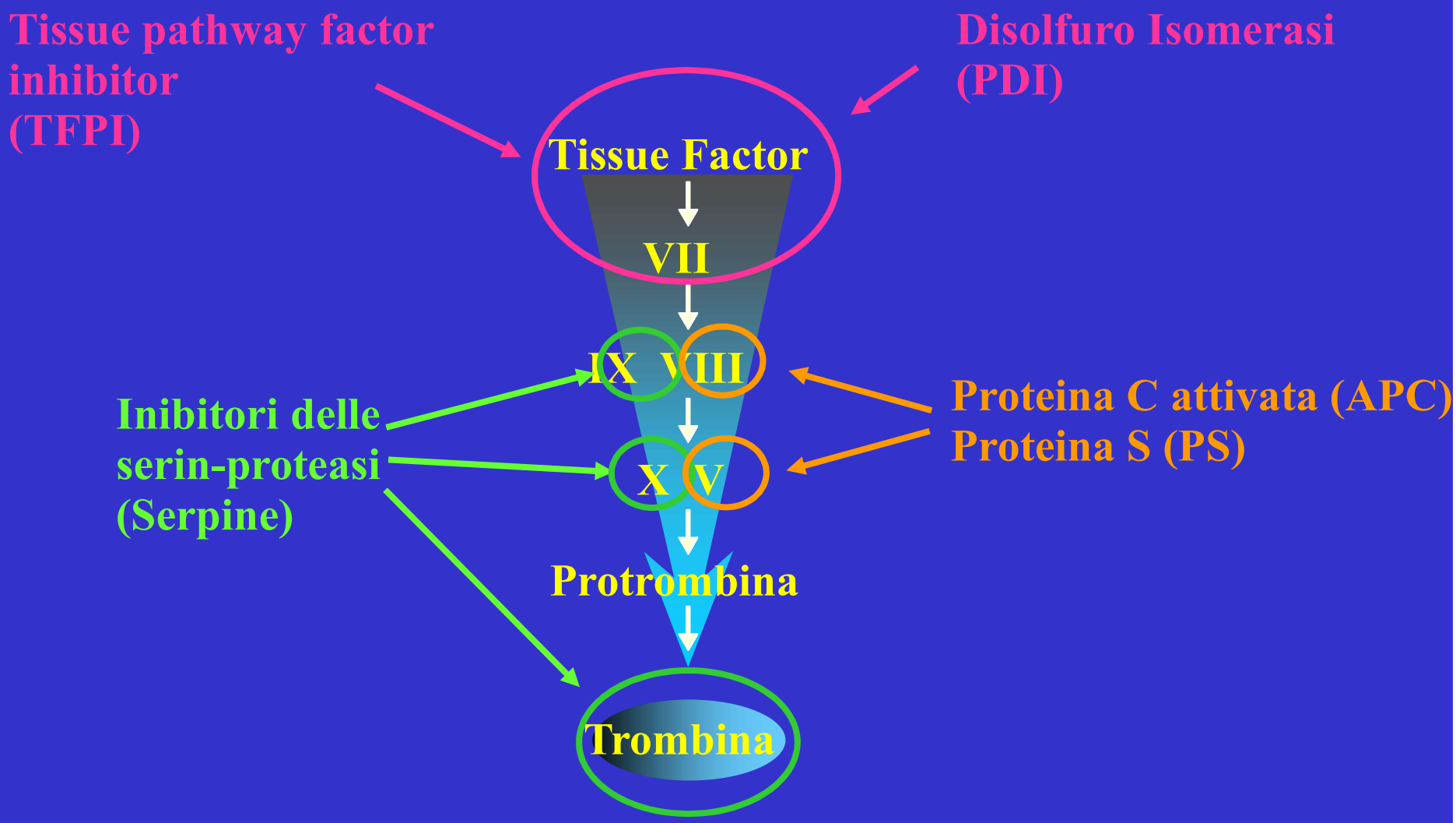


Ds Unnatural Base

Structures of the unnatural Ds–Px and natural A–T and G–C pairs



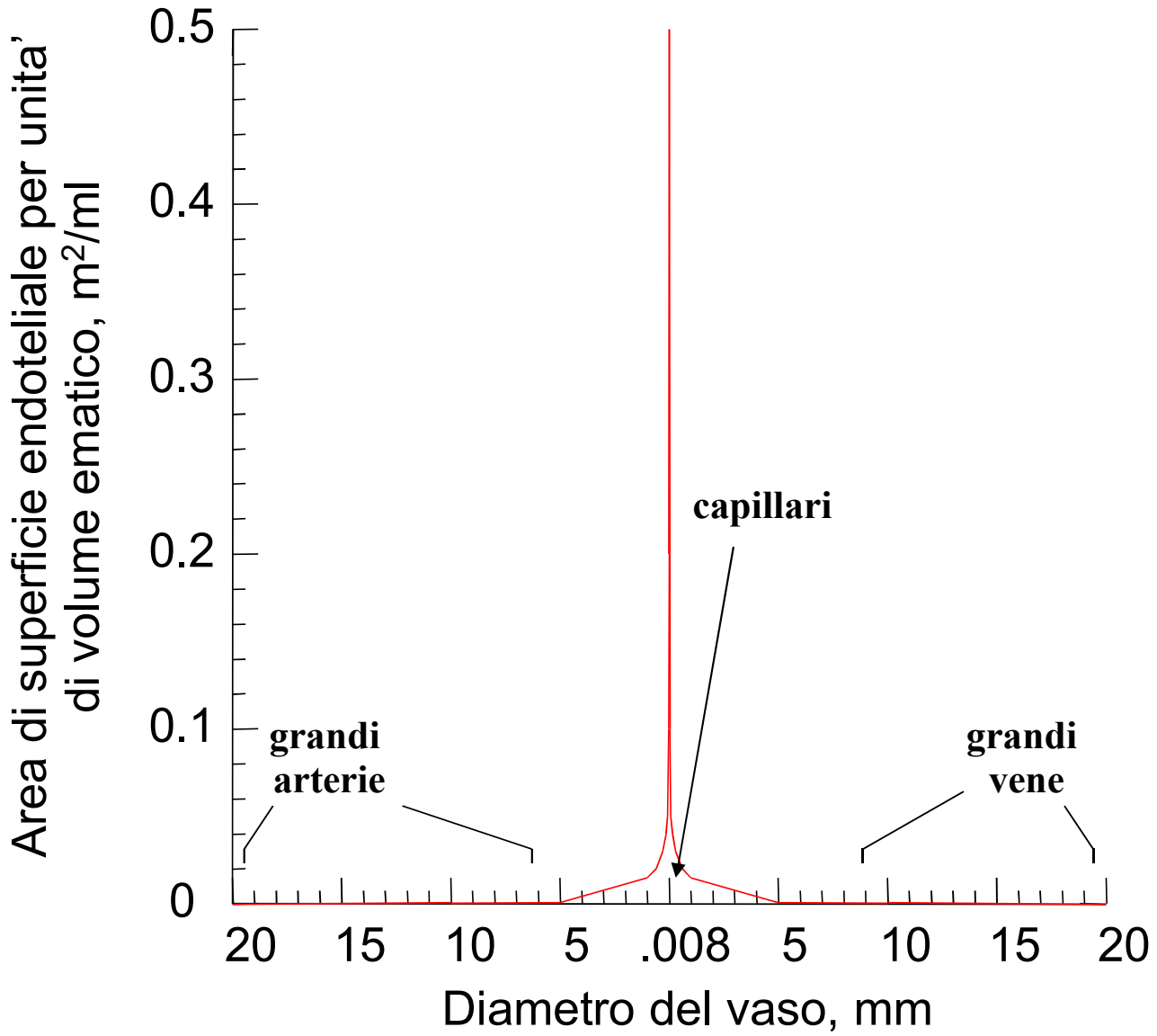
Cascata coagulativa
Regolazione Naturale



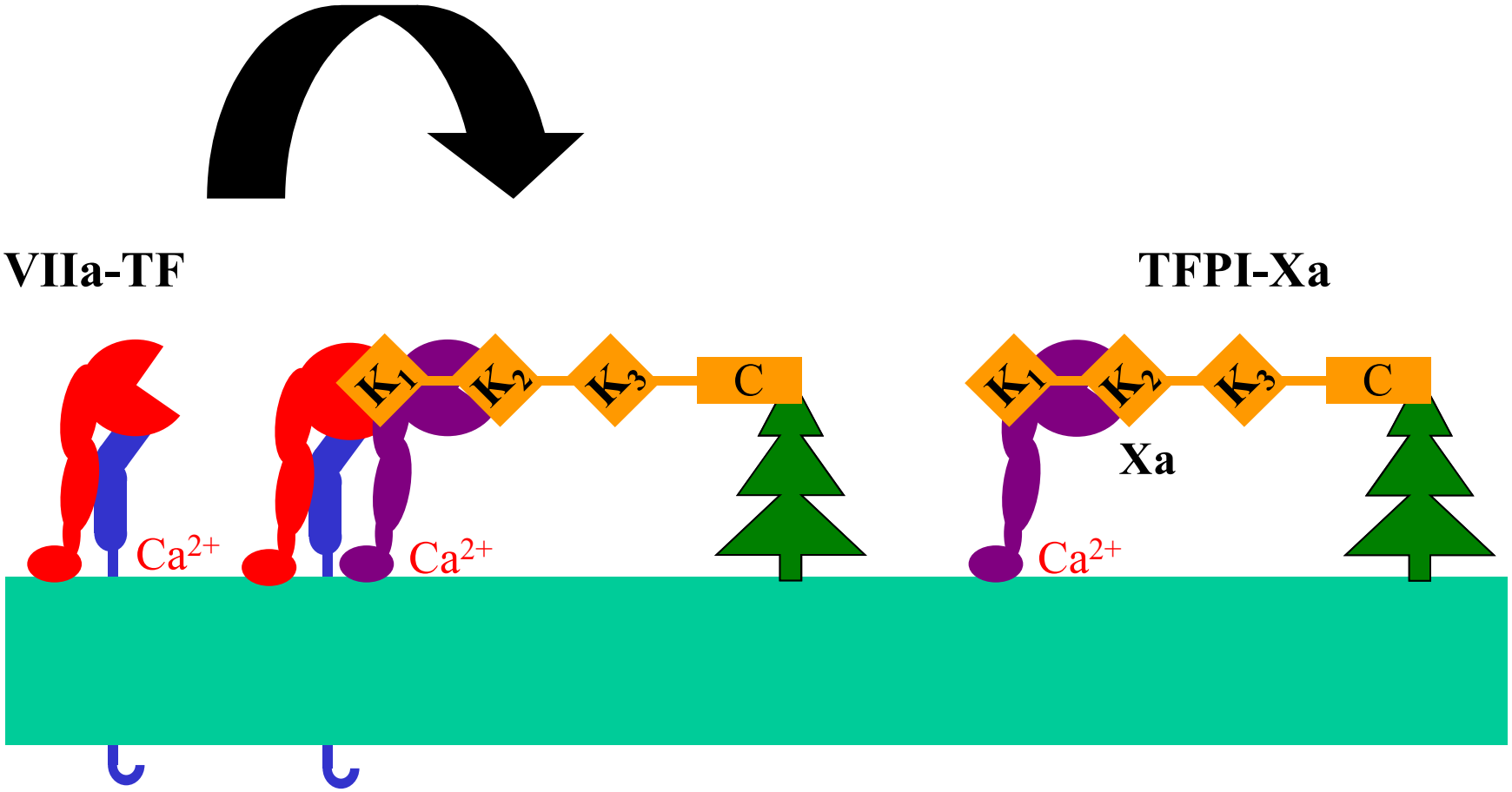
SISTEMI ANTICOAGULANTI NATURALI

Effettore	Target
Inibitore del fattore tissutale (TFPI)	FVIIa-FT
Sistema Antitrombina-eparina	Enzimi (XIIa, XIa, IXa, Xa, IIa, VIIa)
Sistema della Proteina C	Cofattori attivati (VIIIa, Va)

Rapporti tra superficie endoteliale e sangue circolante



TFPI-Xa, inibitore di VIIa-FT



DNA

A DNA template was synthesized with the sequence 5′-GGAGGGAAAAGTTATCAGGC-N40-GATTAGTTTTGGAGTACTCGCTCC-3′

“N40” =40-nucleotide sequence in which there is an equal probability of incorporating a dA, dC, dG, or dT residue at each position and
“d” = 2′-H residue

The DNA template was amplified by polymerase chain reaction (PCR) with forward primer 5′-GACTGTAATACGACTCACTATAGGAGGGAAAAGTTATC-AGGC-3′ and reverse primer 5′-GGAGCGAGTACTCCAAAACTAATC-3′

RNA -selection

- Transcribed to generate a starting pool of approximately 10^{14} different sequences comprised of mA, mG, and mU residues,

"m" = 2′-OCH3 residue

11 rounds of selection were carried out by first incubating the pool of molecules with recombinant full-length TFPI The round 11 pool was cloned and sequenced.

- Individual clones were generated by chemical synthesis
- Clones were tested for

  binding to recombinant TFPI with a nitrocellulose dot blot binding assay

  and for inhibition of TFPI

the clone

(5′-mGmGmAmGmGmGmAmAmAmAmGmUmUmA-
mUdCmAmGmGdCdCmUmGmAmAmUmUmUmGmGmAmAmUmAmUmA
dCmUmUmGmGdCmUdCmGmUmUmAmGmGmUmGdCmGmUmAmUmA
mUmAmGmAmUmUmAmGmUmUmUmUmGmGmAmGmUmAdCmUdCmG
dCmUdCdC-3′)

was determined to bind to TFPI with nanomolar affinity and inhibit its activity in plasma at nanomolar concentrations.

Synthesis modification

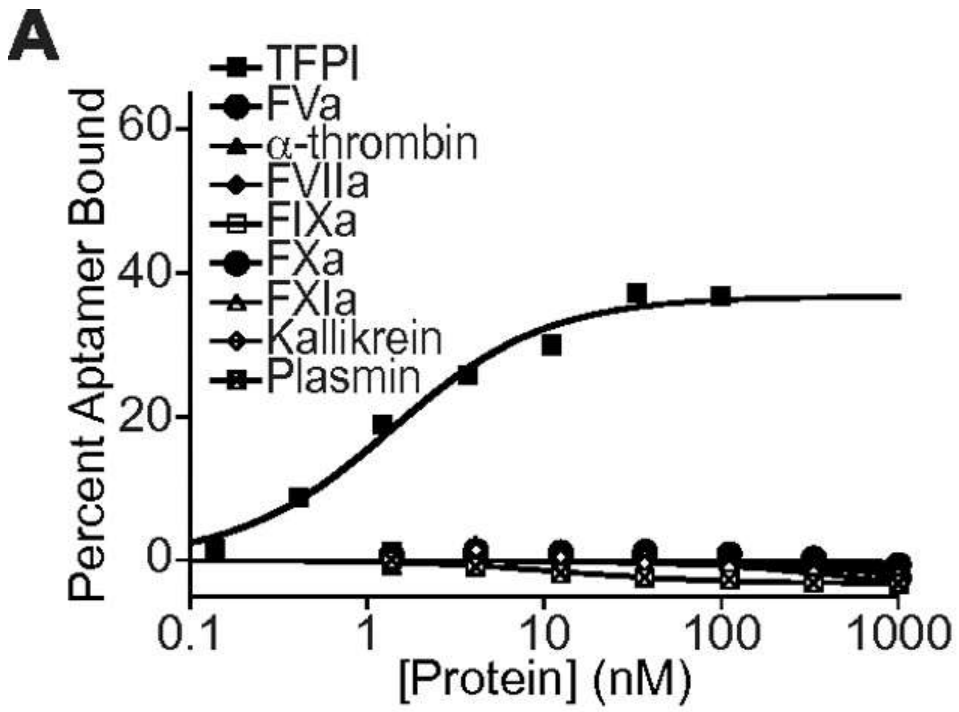
- The core aptamer motif, ARC17480, was identified by design of molecules that contained a portion of the parent clone sequence and evaluation in the same assays.

- The aptamer was synthesized with a hexylamine linker $-CH_3(CH_2)_5NH_2-$ at the 5′-end

- which was conjugated postsynthetically to a branched 40 kDa PEG moiety - $(HO-CH_2-(CH_2-O-CH_2-)_n-CH_2-OH$ - to give rise to ARC19499.

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

ARC17480 binding to TFPI and other proteins.



Waters E K et al. Blood 2011;117:5514-5522

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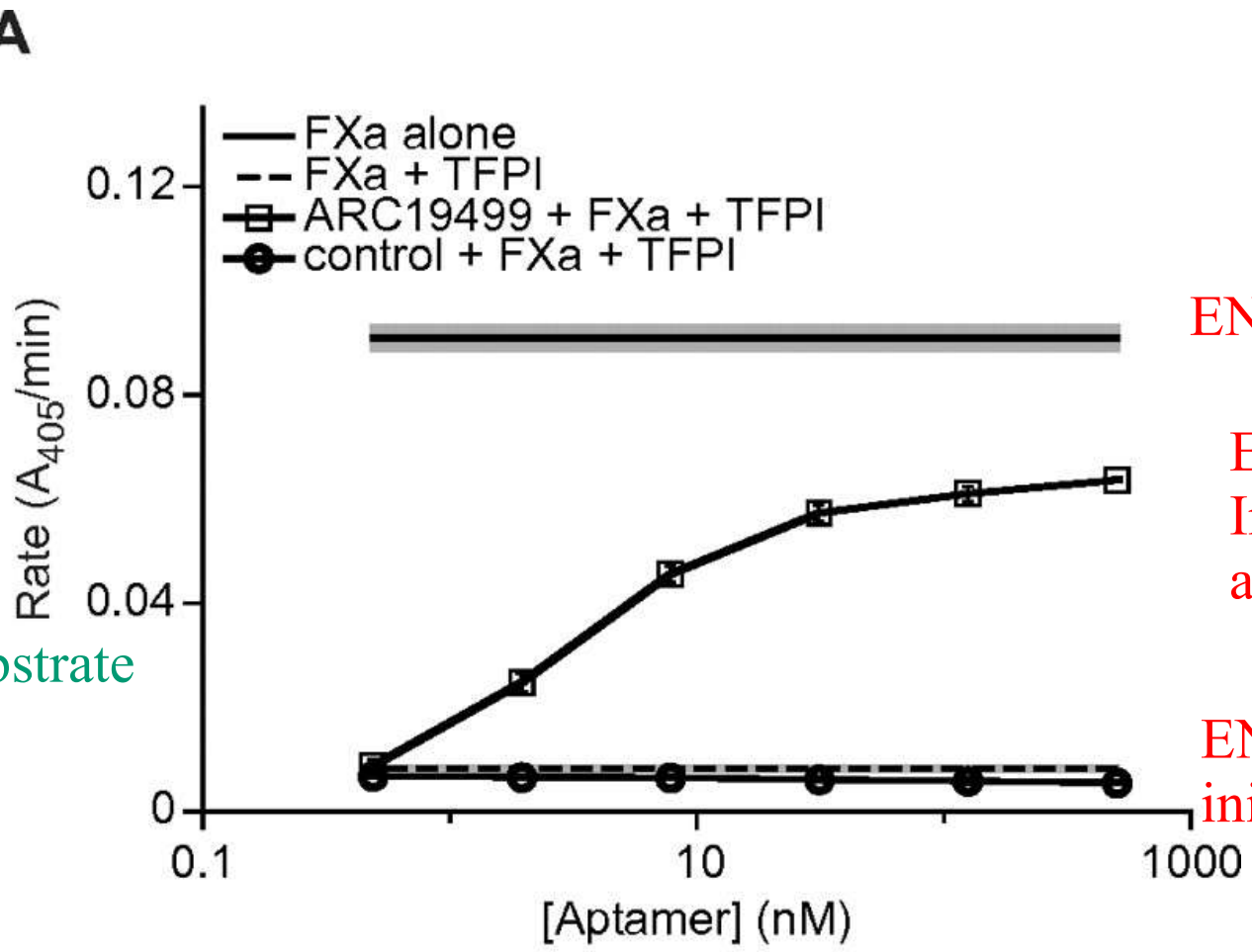
Activity of ARC19499 in TFPI-dependent assays using purified proteins.



ENZIMA

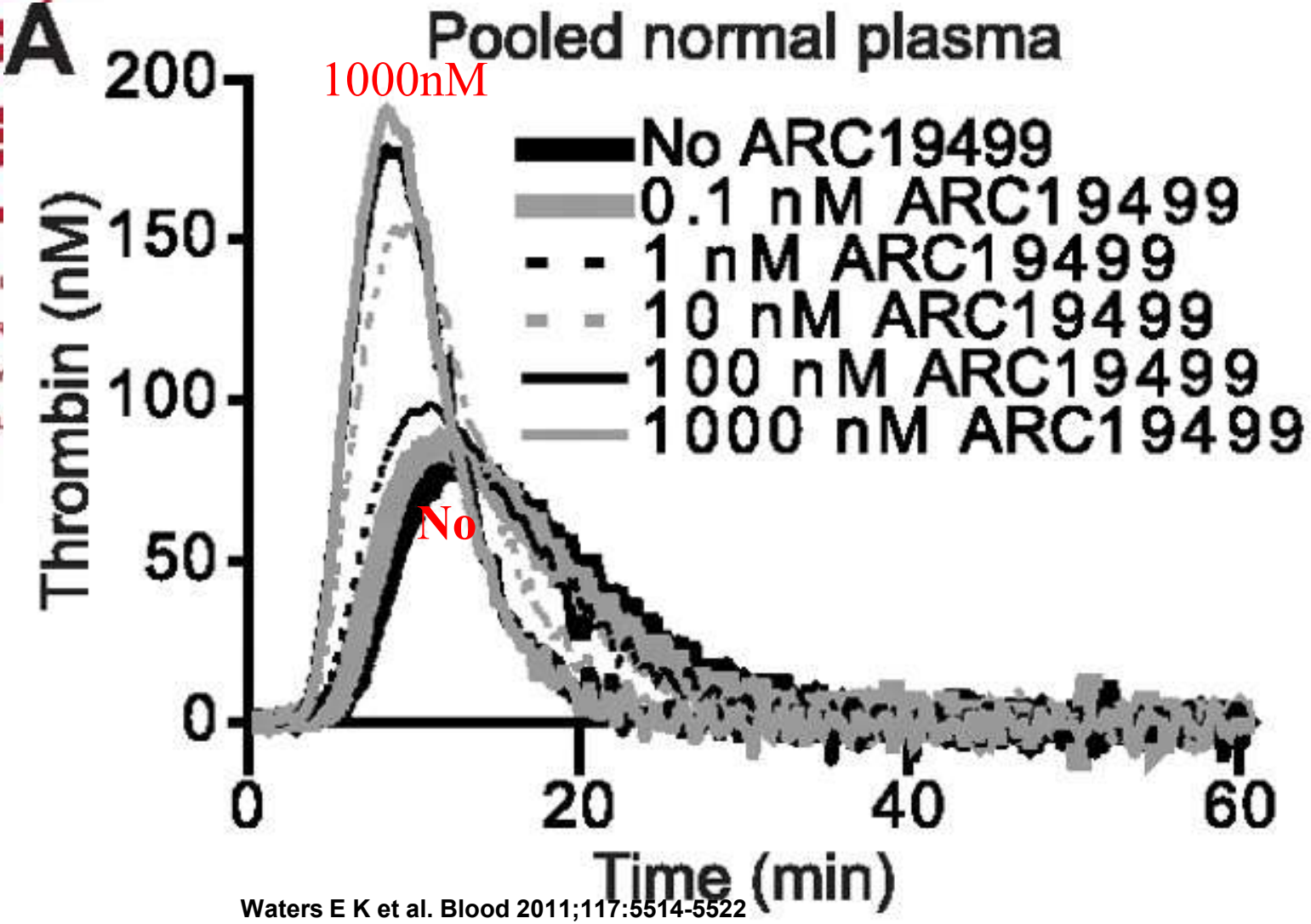
ENZIMA+ Inibitore+ aptamero

ENZIMA+ inibitore

chromogenic substrate

Waters E K et al. Blood 2011;117:5514-5522

ARC19499 inhibition of TFPI in human plasma.



Waters E K et al. Blood 2011;117:5514-5522

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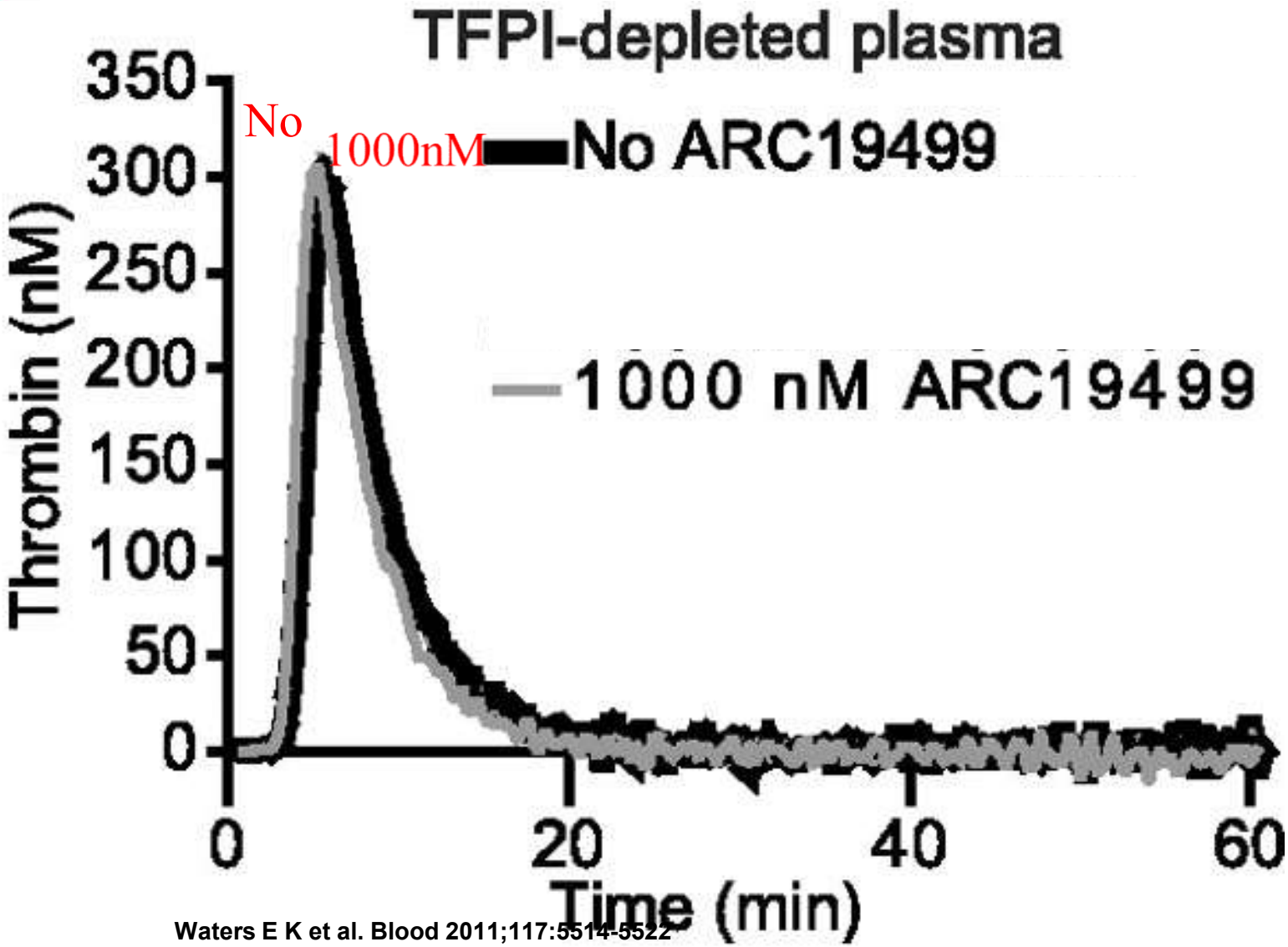
ARC19499 inhibition of TFPI in human plasma.



Waters E K et al. Blood 2011;117:5514-5522

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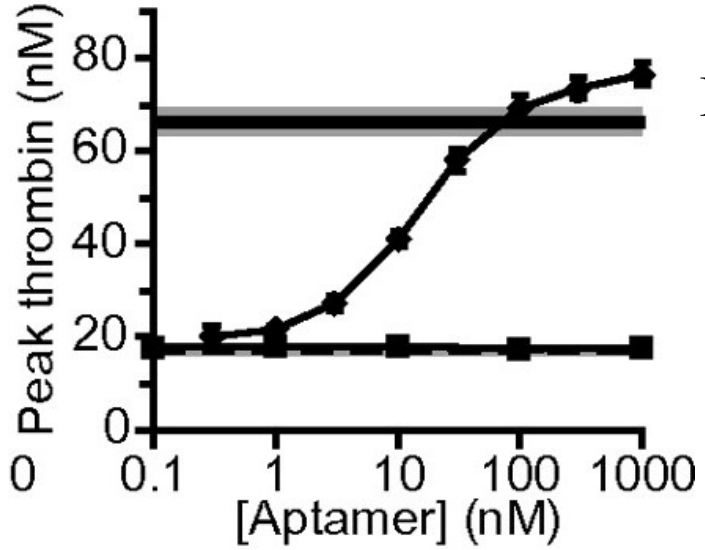
ARC19499 effect on thrombin generation in human plasma.

Activity in hemophilia A plasma



Hemophilia A + aptamer

normal plasma

Hemophilia A

Normal plasma (solid lines)
Hemophilia (dashed lines)

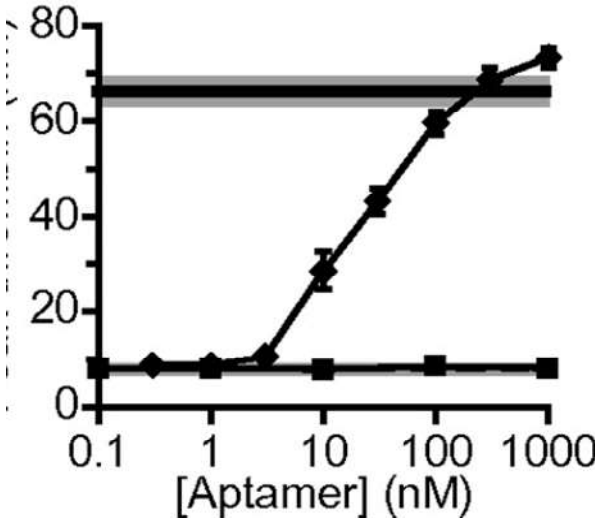
+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

ARC19499 effect on thrombin generation in human plasma.

Activity in hemophilia B plasma



Hemophilia B+ aptamer
normal plasma

Hemophilia B

Normal plasma (solid lines)
Hemophilia (dashed lines)

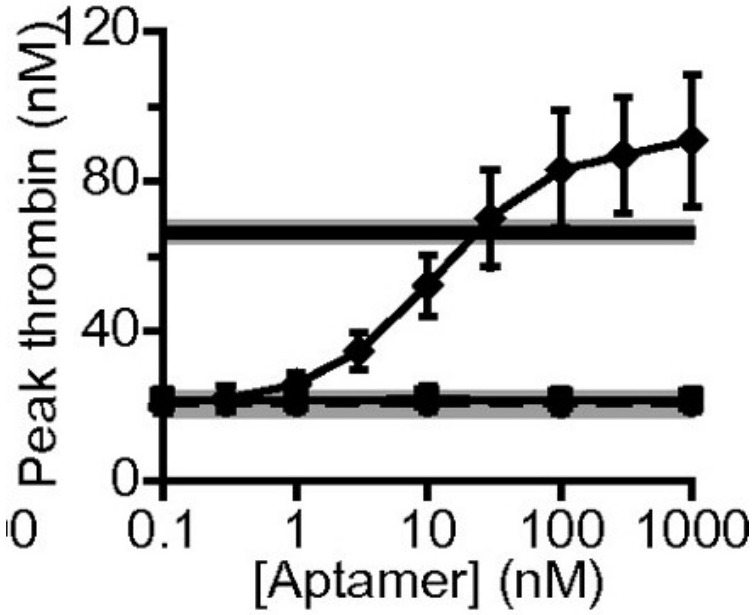
+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

ARC19499 effect on thrombin generation in human plasma.

Activity in plasma with antibody inhibitor



Anticorpo inibitore + aptamer

normal plasma

Anticorpo inibitore

Normal plasma (solid lines)
Hemophilia (dashed lines)

+ ARC19499 (♦)
+ negative control oligonucleotide (■).

Waters E K et al. Blood 2011;117:5514-5522