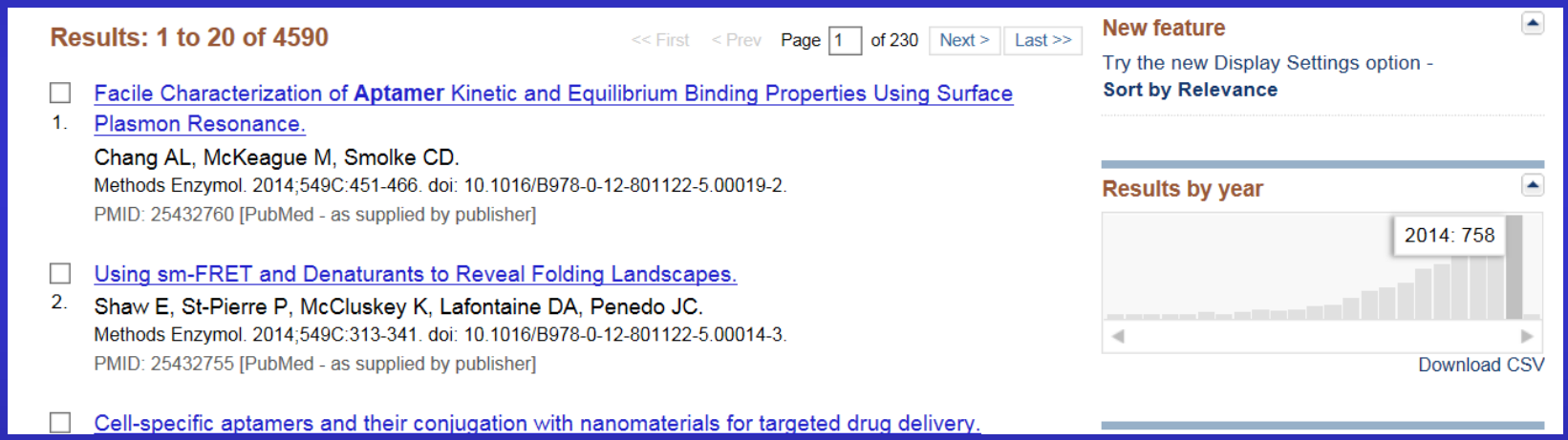
Gli APTAMERI

Aptamers



Results: 1 to 20 of 4590 << First < Prev Page 1 of 230 Next > Last >>

1. Facile Characterization of **Aptamer** Kinetic and Equilibrium Binding Properties Using Surface Plasmon Resonance.
   Chang AL, McKeague M, Smolke CD.
   Methods Enzymol. 2014;549C:451-466. doi: 10.1016/B978-0-12-801122-5.00019-2.
   PMID: 25432760 [PubMed - as supplied by publisher]

2. Using sm-FRET and Denaturants to Reveal Folding Landscapes.
   Shaw E, St-Pierre P, McCluskey K, Lafontaine DA, Penedo JC.
   Methods Enzymol. 2014;549C:313-341. doi: 10.1016/B978-0-12-801122-5.00014-3.
   PMID: 25432755 [PubMed - as supplied by publisher]

Cell-specific aptamers and their conjugation with nanomaterials for targeted drug delivery.

New feature

Try the new Display Settings option - **Sort by Relevance**

Results by year

2014: 758

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Table 1

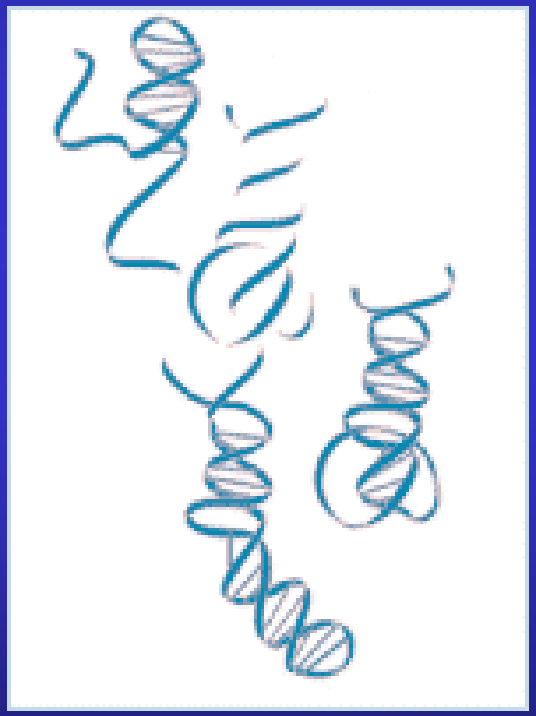
Available aptamers currently under considerations.

Name of the aptamer	Primary target of the aptamer	Status
Macugen	VEGF	Approved [22]
AS1411	Nucleolin	Phase II [25, 26]
REG1	Factor Ixa	Phase II [29, 30]
EYE001	VEGFR	Phase II/III [47, 49]
LY2181308	Survivin mRNA	Phase III [50, 51]
E_2F decoy oligonucleotides	Mesangial cells	Phase III [52, 53]
ARC1779	Vwf	Phase II [31]
NU172	Thrombin	Phase II [32]
E10030	PDGF	Phase II [23]
ARC1905	C5	Phase I [24]
NOX-E36	MCP-1	Phase I [27, 33]
NOX-A12	SDF-1	Phase I [27, 28]
NOX-H94	Hepcidin	Phase I [21]
BAX499/ARC19499	TFPI	Phase I [34, 35]
DNA aptamers	Thrombin	Research [11]
DNA aptamers	Phosphatidylserine (PS)	Research [11, 38] (see Section 2.4)

Biomed Res Int

APTAMERI

Acidi nucleici a singolo filamento caratterizzati da una specifica **struttura tridimensionale** che si lega direttamente alla proteina target.

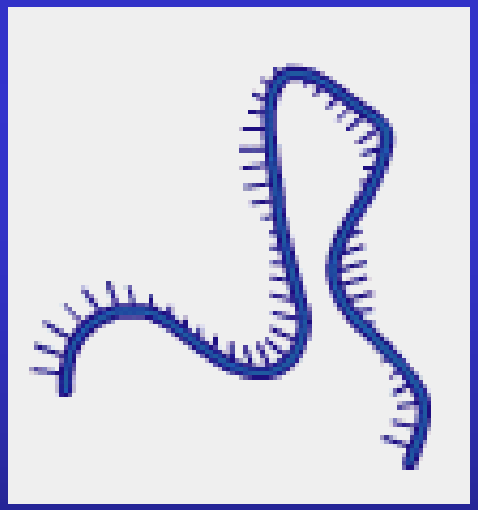


Interazione Acido Nucleico/Proteina

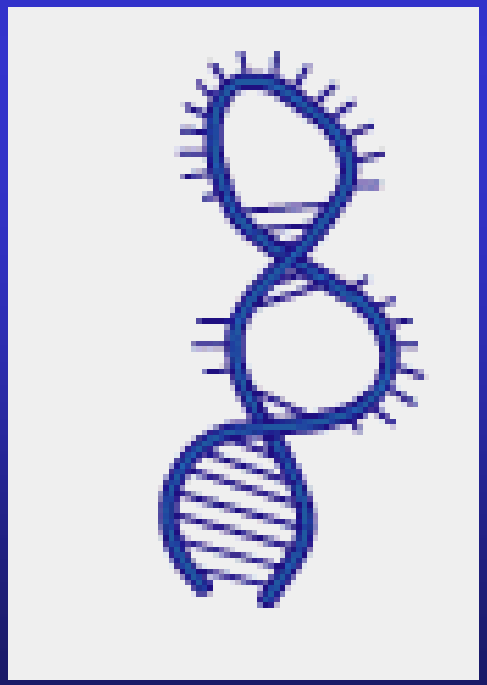
Aptameri

Dimensioni: 30-70 nucleotidi

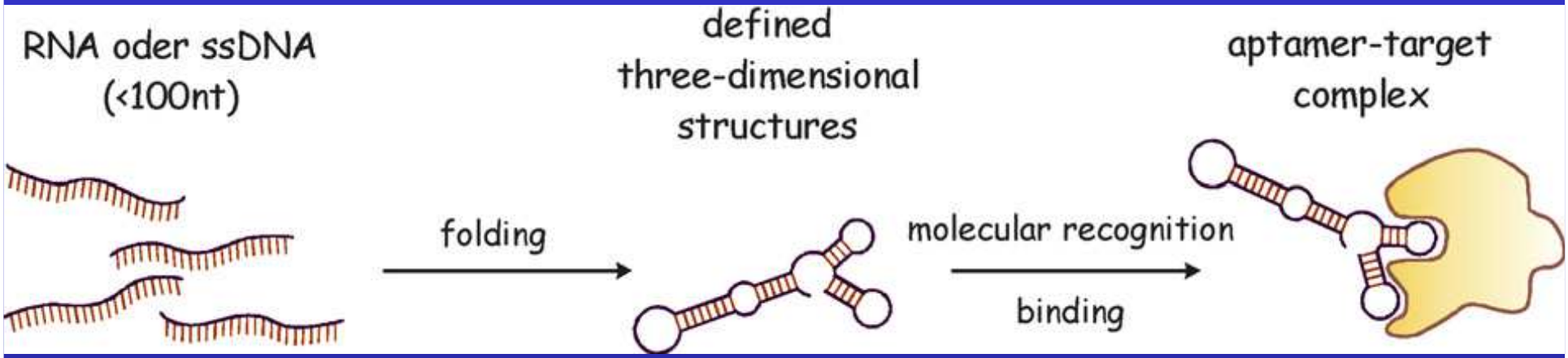
Molecola Lineare



Folding

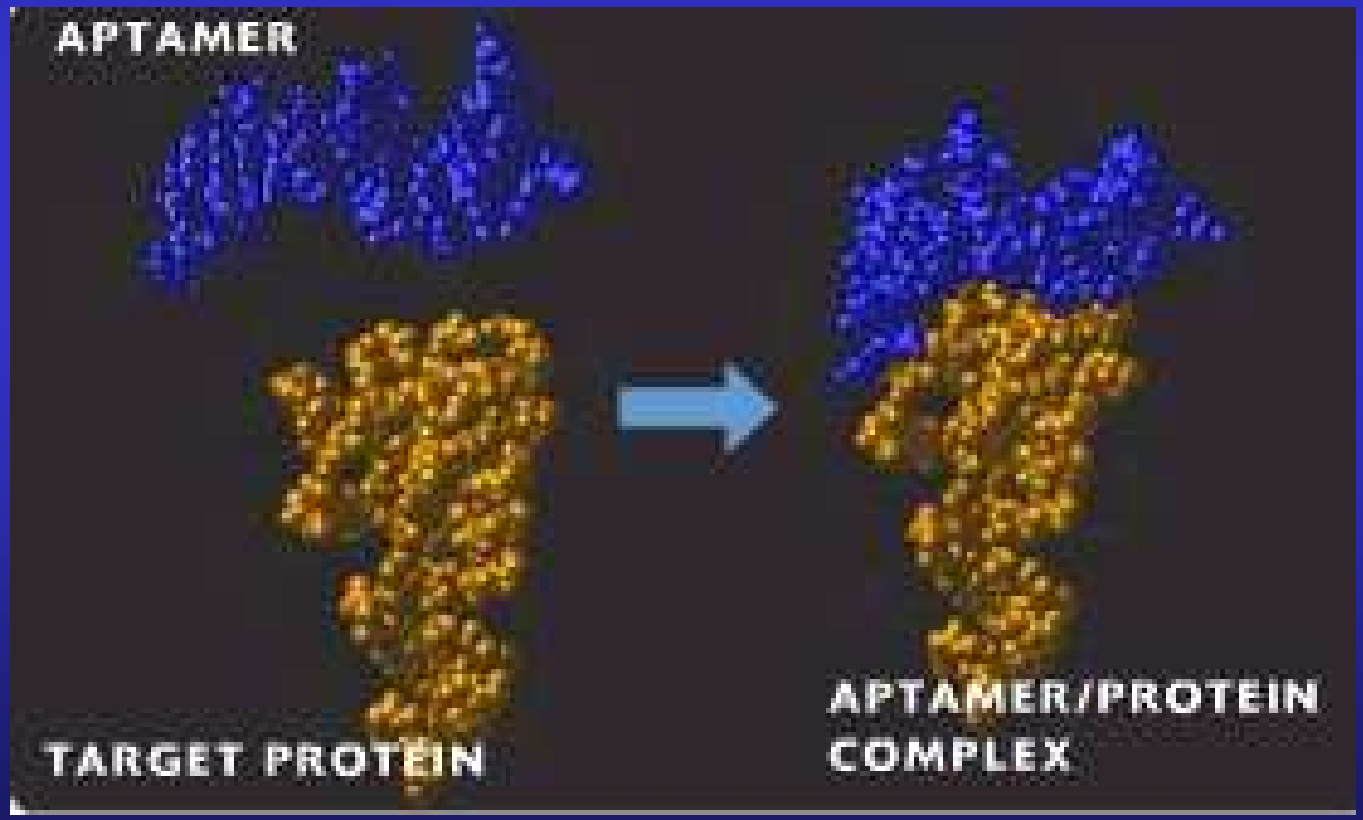


Struttura tridimensionale stabile


RNA oder ssDNA
(<100nt)
folding
defined
three-dimensional
structures
molecular recognition
binding
aptamer-target
complex

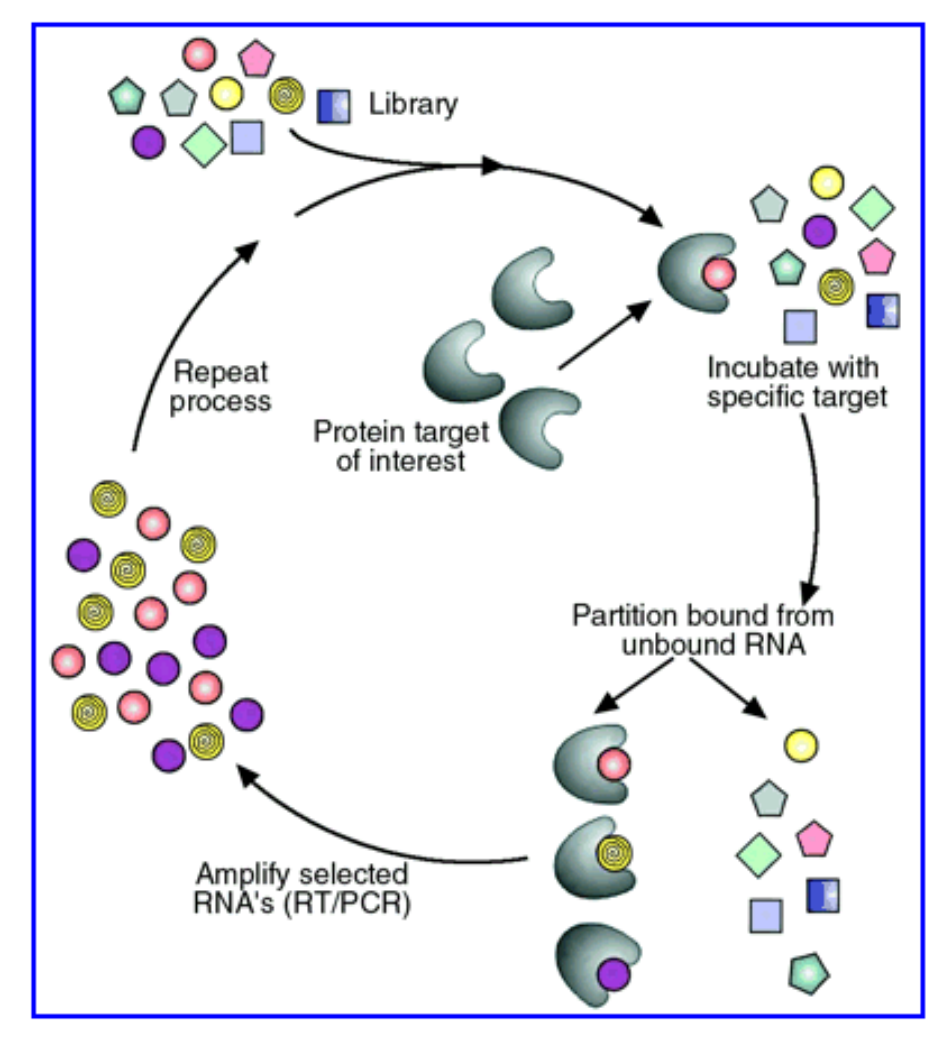
Anatomia degli Aptameri

Gli aptameri sono molecole selezionate per legarsi in modo specifico ad una predefinita *proteina target*



Selezione in vitro degli Aptameri:

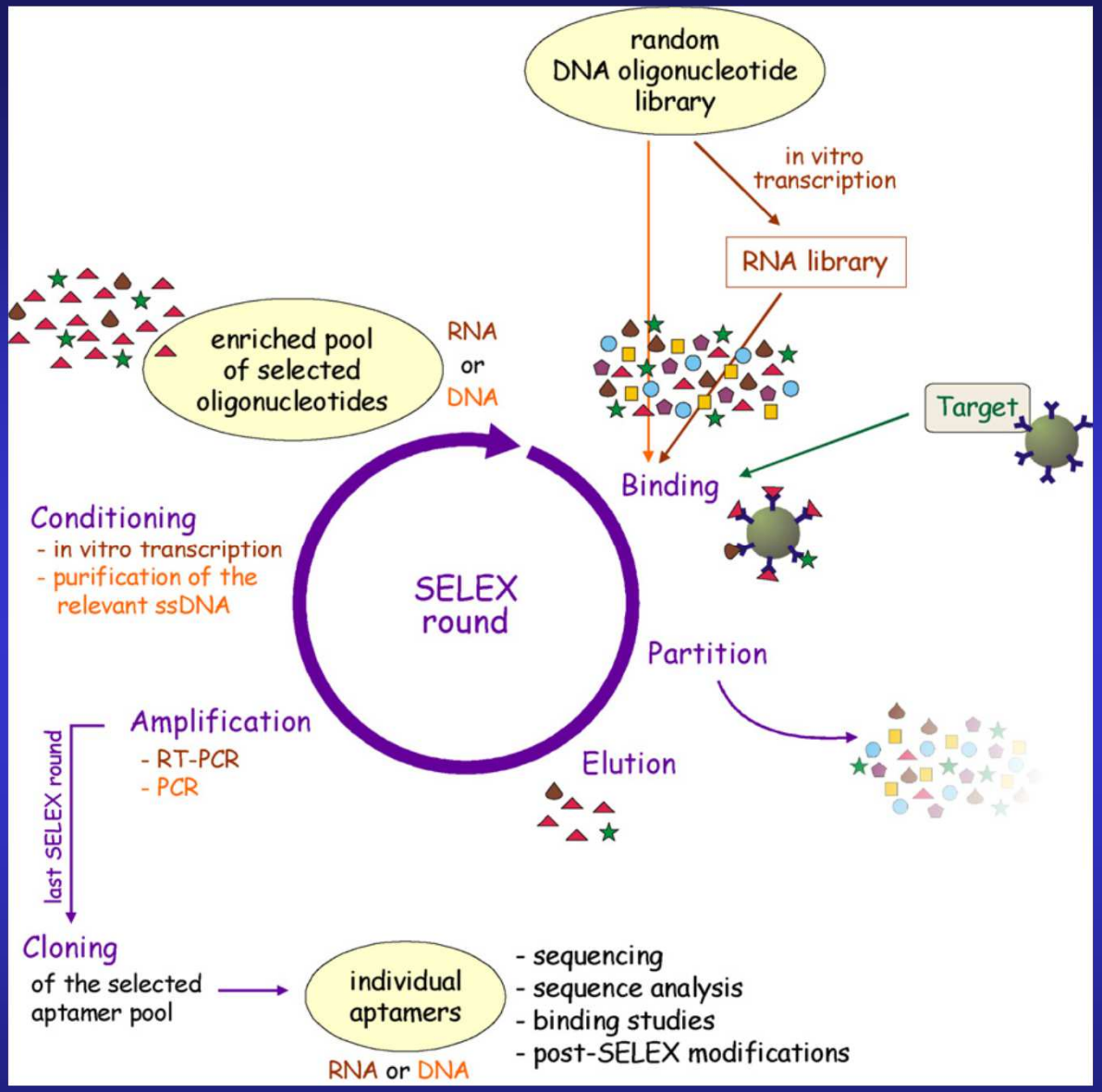
SELEX (systematic evolution of ligands by exponent enrichment)

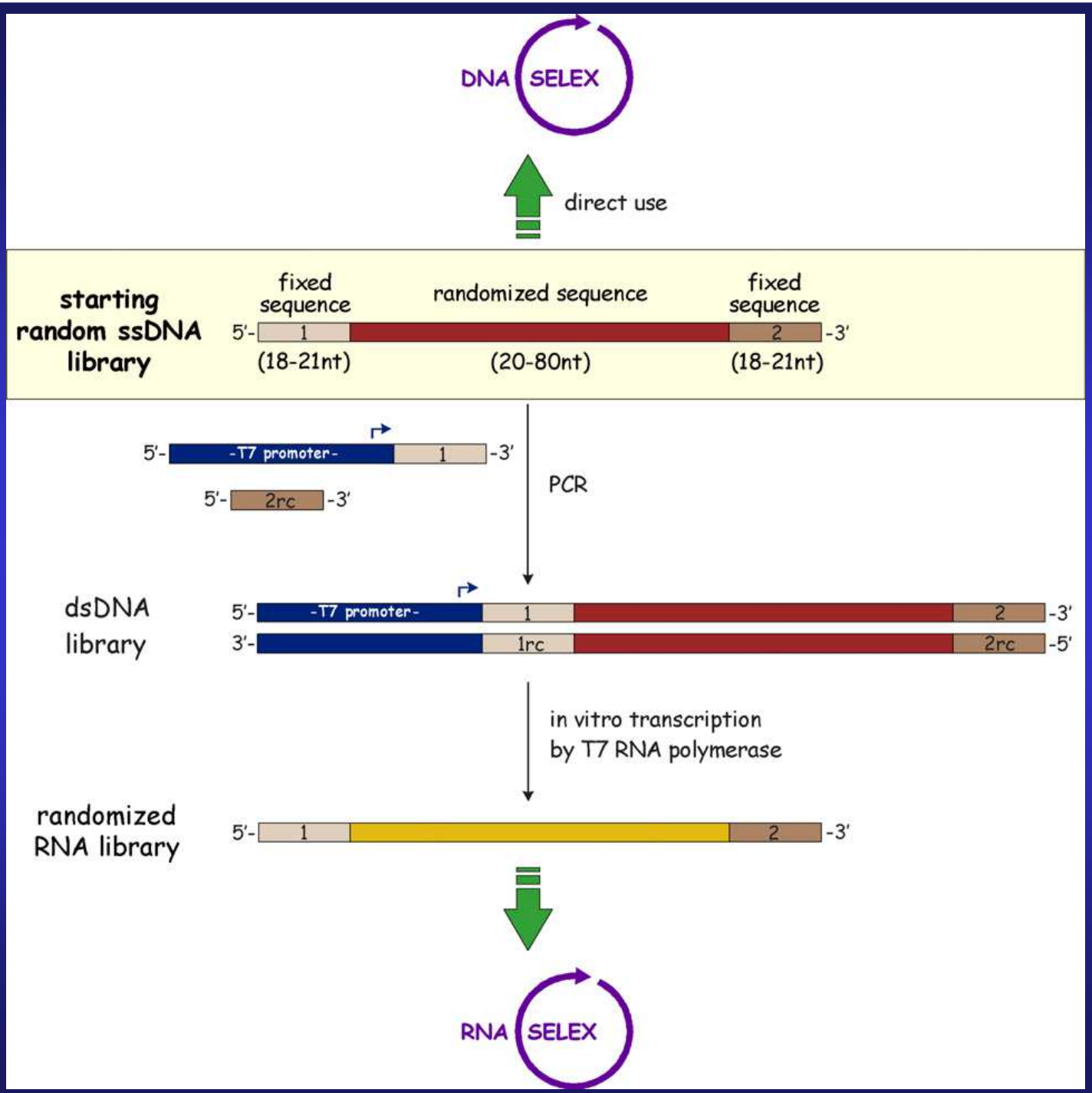


Selezione in vitro degli Aptameri:

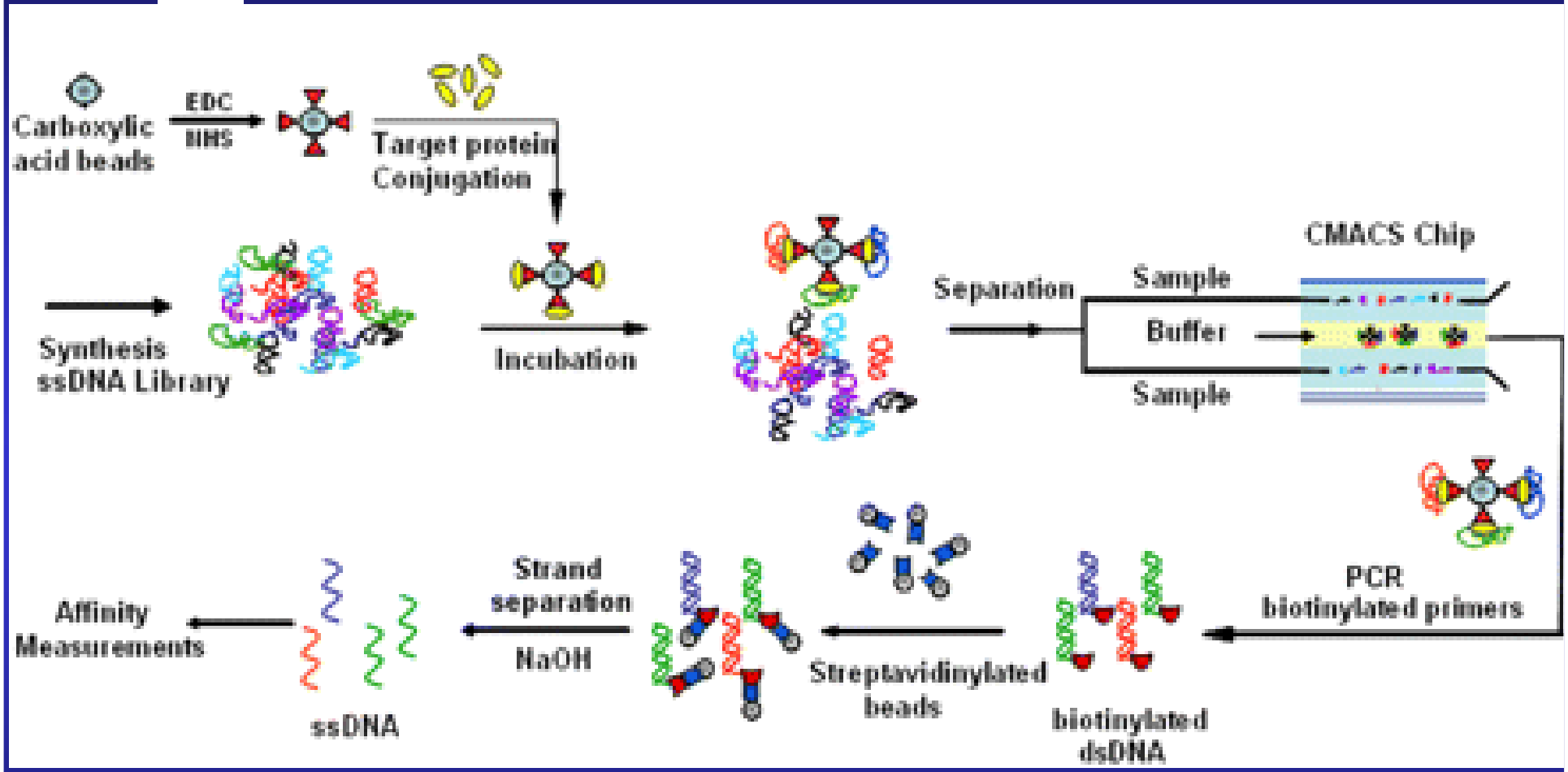
SELEX (systematic evolution of ligands by exponent enrichment)

1. Sintesi chimica di 10^{14} RNA o DNA (Libreria)
2. Incubazione con la proteina target: cromatografia per affinità
3. Rimozione degli oligo *non legati* mediante buffer di lavaggio
4. Rimozione degli oligo *legati* alla proteina target con una soluzione contenente la proteina target
5. Retrotrascrizione e PCR (RNA) o solo PCR (DNA) degli oligo che si sono legati
6. Trascrizione in vitro (RNA) o solo denaturazione (DNA) per separare i filamenti
7. Inizio di un nuovo ciclo fino a 5-10 cicli


random
DNA oligonucleotide
library
in vitro
transcription
RNA library
enriched pool
of selected
oligonucleotides
RNA
or
DNA
Target
Binding
Conditioning
- in vitro transcription
- purification of the
relevant ssDNA
SELEX
round
Partition
Amplification
- RT-PCR
- PCR
last SELEX round
Elution
Cloning
of the selected
aptamer pool
individual
aptamers
RNA or DNA
- sequencing
- sequence analysis
- binding studies
- post-SELEX modifications


DNA SELEX
direct use
starting random ssDNA library
fixed sequence
randomized sequence
fixed sequence
5'-
1
2
-3'
(18-21nt)
(20-80nt)
(18-21nt)
5'-
-T7 promoter-
1
-3'
5'-
2rc
-3'
PCR
dsDNA library
5'-
-T7 promoter-
1
2
-3'
3'-
1rc
2rc
-5'
in vitro transcription
by T7 RNA polymerase
randomized RNA library
5'-
1
2
-3'
RNA SELEX

Automazione SELEX



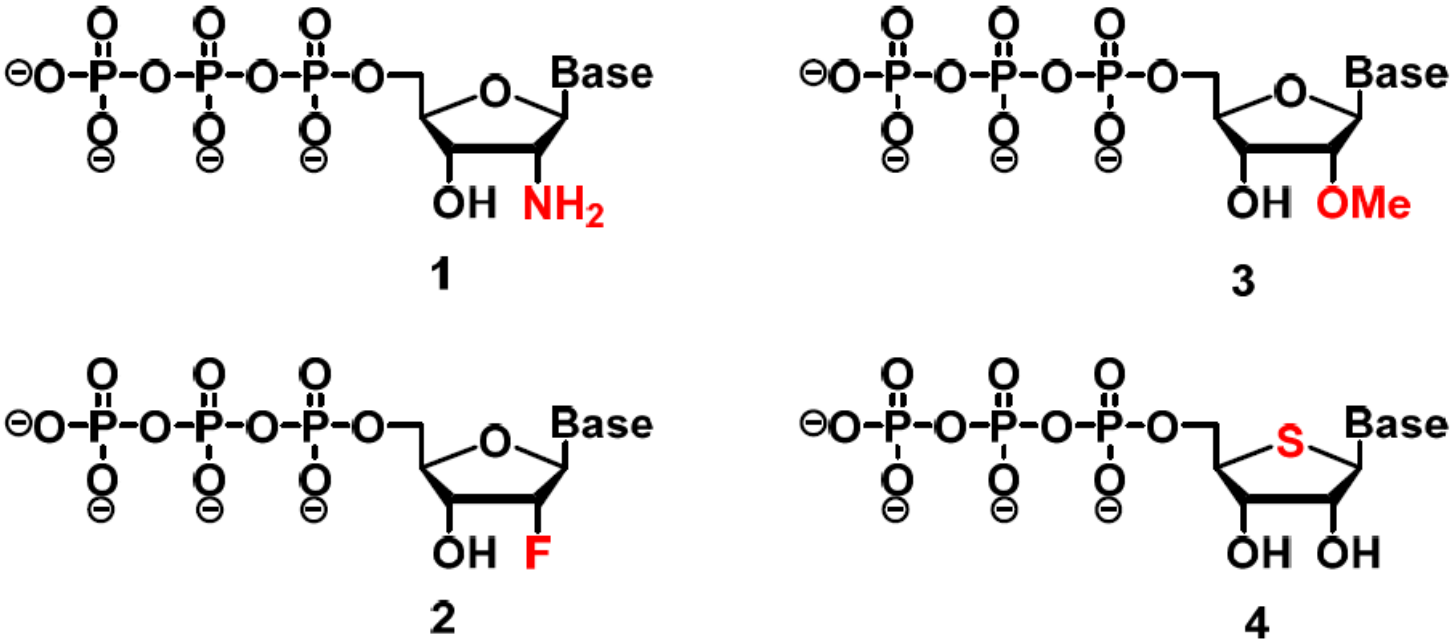


Figure 2. Chemical structures of 2′-modified nucleotides used in selection experiments to generate aptamers with enhanced pharmacokinetic properties: 2′-amino-NTPs **1**, 2′-fluoro-NTPs **2**, 2′-methoxy-NTPs **3**, and 4′-thio-NTPs **4**.

Table 1. Summary of the recently generated aptamers using the 2′-fluoro modificat

Aptamer Name	Aptamer Target	K_d Value (nM)
E07	Epidermal growth factor receptor (EGFR)	2.4
CL4	Epidermal growth factor receptor (EGFR)	10
S2	Prostate-specific antigen (PSA)	630
A15	Brain penetrating aptamer	-
R-F t2	NS5B replicase, essential for the replication of hepatitis C virus (HCV)	2.6
Gint4.T	Platelet-derived growth factor receptor β (PDGFRβ)	9.6
GL21.T	Transmembrane tyrosine kinase receptor (RTK) Axl	12
G-3	C-C chemokine receptor type 5 (CCR5)	110
C26-50	*N*-methyl-D-aspartate (NMDA) receptor ion channel	120
Apt1	CD44, a cell-surface glycoprotein that serves as a cancer stem cell marker	81.3
B-68	HIV-$1_{Ba\text{-}L}$ glycoprotein 120	52
GL44	Human U87MG glioma cells	38
RNA 14-16	p68 RNA helicase, which is involved in colorectal cancer	13,8
FAIR-6	Interleukin-6 receptor (IL-6R)	40.9
CD28Apt2, CD28Apt7	CD28 costimulatory receptor for the activation of T lymphocytes	40, 60
9C7	OX40 costimulatory receptor	1.7
αV-1, β3-1	αV and β3 subunits of integrin αVβ3	2.7, 6.5

Applicazioni degli Aptameri:

1. Ricerca

2. Diagnostica

3. Terapia

Applicazioni degli Aptameri:

ALTERNATIVA AGLI ANTICORPI

- elevate specificità e affinità unite a ridotte dimensioni

- sintesi chimica (vs sintesi in animali o colture cellulari)

- facilmente modificabili: marcatura con radioattivo, code fluorescenti e biotinilate...

APPLICAZIONI IN VIVO:

- nessuna tossicità dimostrata (facilmente eliminabili da sangue e reni)
- non immunogenici
- tessuto-specifici

Table 3
Examples of applied aptamers

Target	Aptamer/assay	Field of application	Research or product state	Reference
VEGF	RNA aptamer, chemically modified	Therapy, wet age related macula degeneration	Product: Macugen® (Pfizer Inc.)	Maberley (2005) and Chapman and Beckey (2006)
Thrombin	DNA aptamer (thrombin inhibitor ARC-138)	Therapy, anticoagulant	Product development, Phase 1 studies in August 2004 (Archemix Corp.)	Nimjee et al. (2005a)
Factor IXa	RNA aptamer (factor IXa inhibitor) and its antidote REG1	Therapy, anticoagulant	Product development, Phase 1 studies completed in 2006, (Regado Biosciences Inc.; Archemix Corp.)	Nimjee et al. (2005a) and Dyke et al. (2006)

TERAPIA:

Condizioni patologiche acute e spazialmente confinate

- **Trombosi: aptameri contro trombina, FVIIa, FIXa**

- **Cancro: aptameri contro proteine segnale (es. Crescita, differenziazione, trasformazione cellulare...)**

- **Patologie virali: identificazione e inibizione di proteine virali**

Gli APTAMERI: un'applicazione

Rusconi CP, Scardino E, Layzer J, Pitoc GA, Ortel TL, Monroe D, Sullenger BA

RNA aptamers as reversible antagonists of coagulation factor IXa

Nature 2002; 419: 90-94

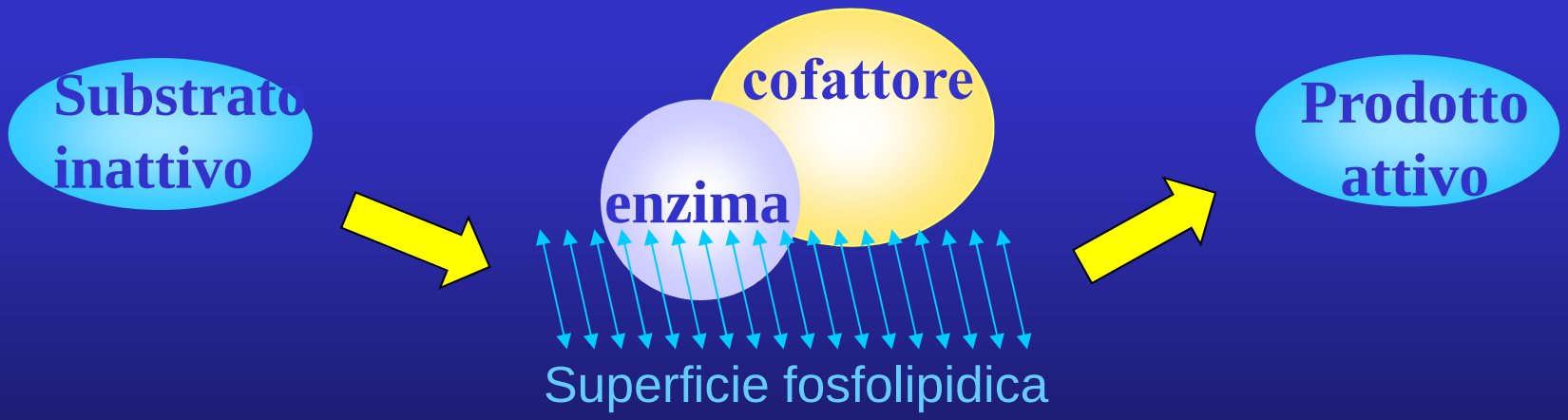
(www.nature.com)

COAGULAZIONE DEL SANGUE

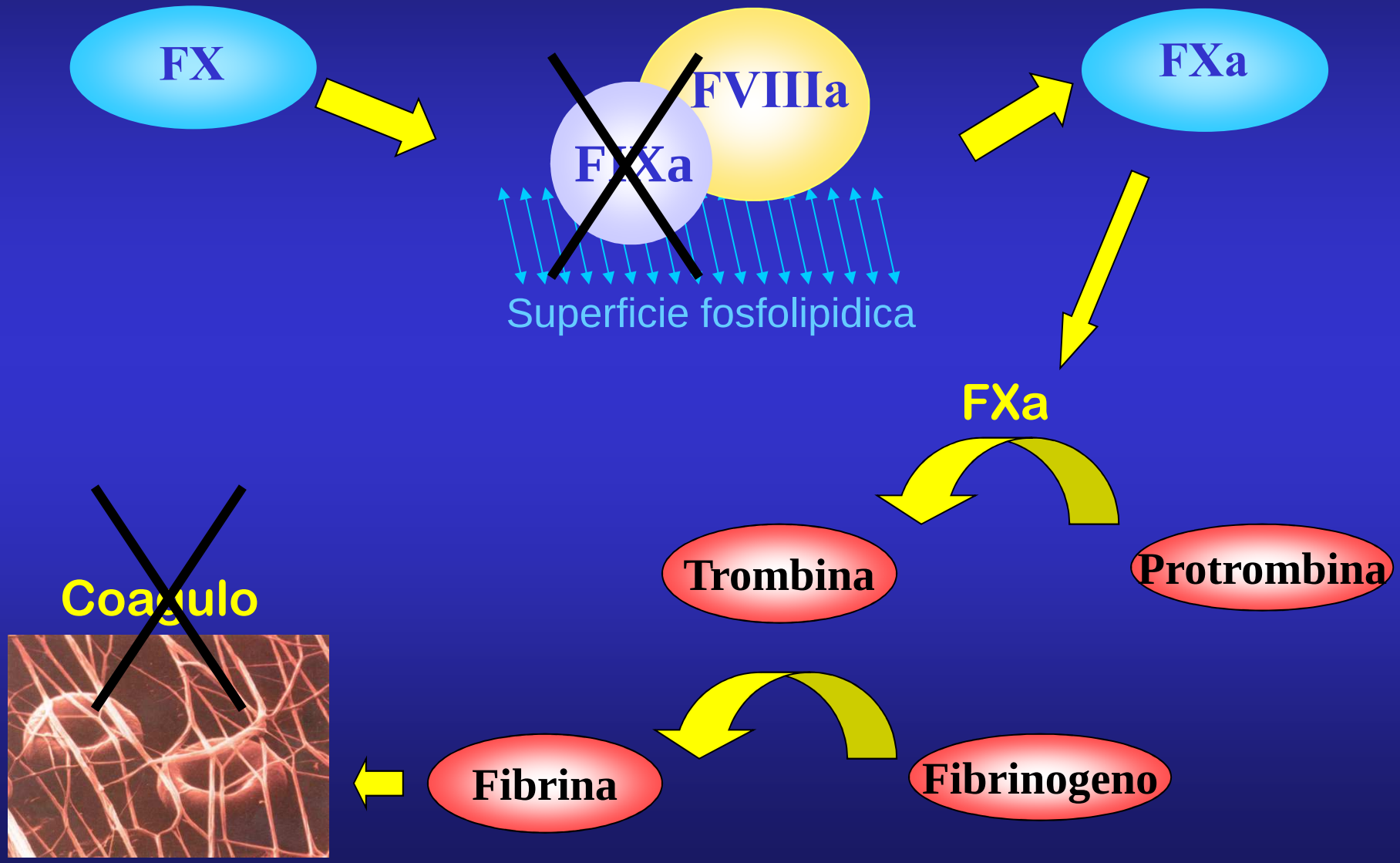
Danno vascolare

Attivazione a cascata di fattori e cofattori plasmatici

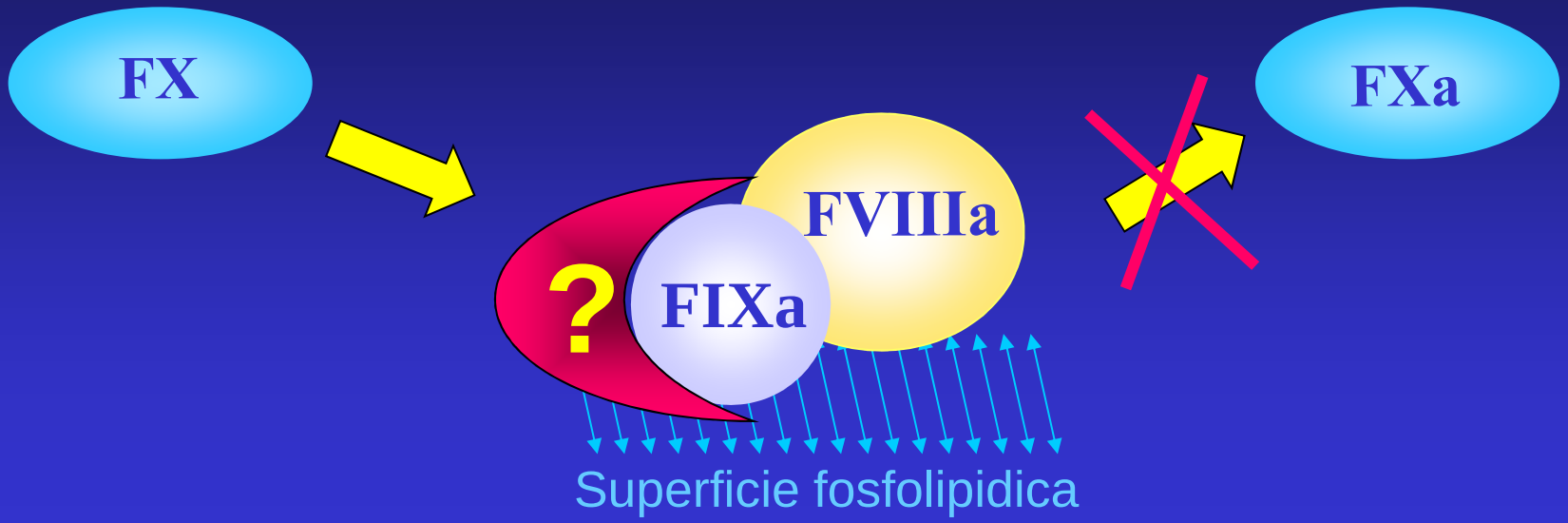
Complessi macromolecolari

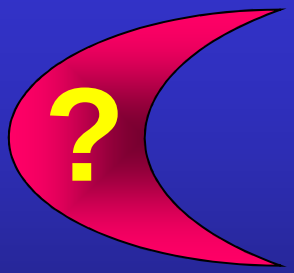


Complesso di attivazione del FX



Complesso di attivazione del FX





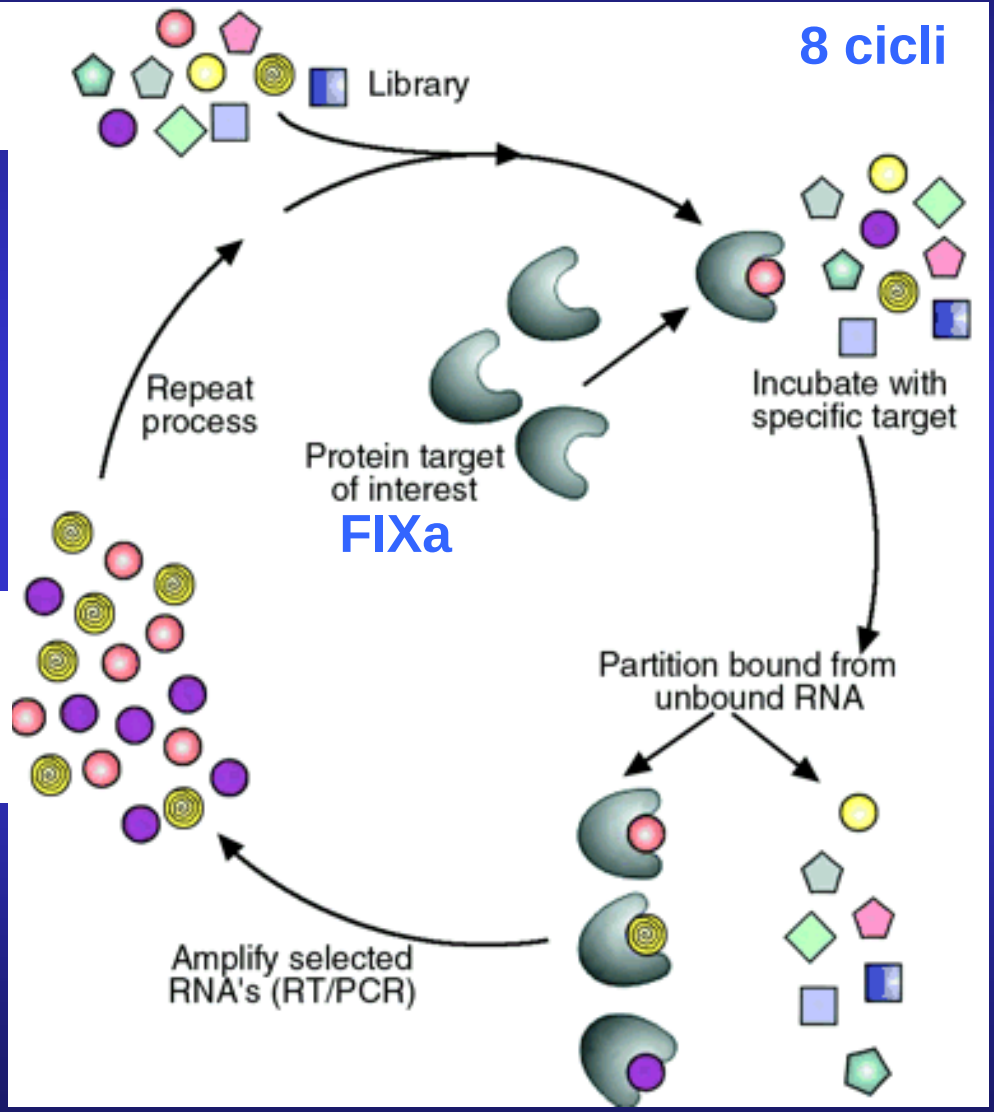
Aptamero selezionato

1. Elevata AFFINITA' con il FIXa
2. SPECIFICITA' per il FIXa

Selezione degli Aptameri
SELEX

Libreria: 10^{14} oligonucleotidi (RNA)

8 cicli



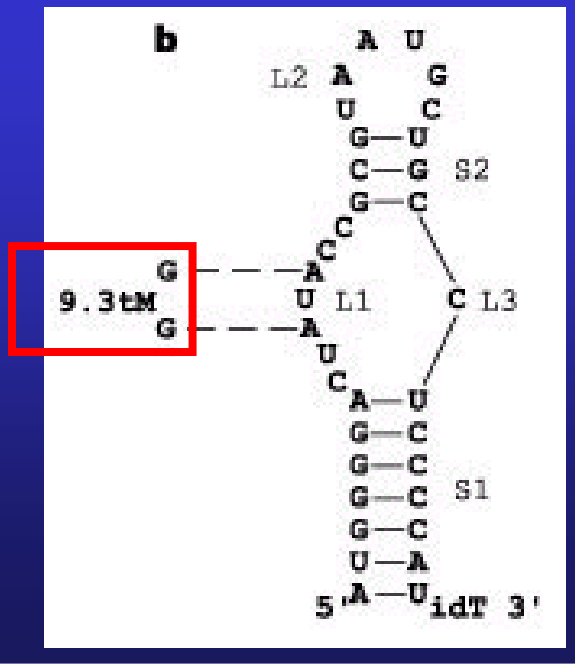
Retrotrascrizione degli RNA selezionati e sequenziamento

1. AFFINITA' con il FIXa

Aptamer		S1	L1	S2	L2	S2	L3	S1	
9-3	5'	GGGAUGGGGA	CUAUACC	GCG	UAAUGC	UGC	C	UCCCCAUUCC	GGAACGCU 3'
9-20	5'	GGGGA	CUAUACCG	GCA	AUCG	UGC	A	UCCCC	UGGACCUAACAAUA 3'
9-19	5'	GGaUGGGA	CCAUA	ACGA	CUAC	UCGU	GAA	UCCCACC	AUCAGCGCACAA 3'
9-4	5'	GGGaUGGGC	ACUAUAC	GCA	UCU	UGC	U	GCCUGCCC	GCGAGUCAAUUG 3'
9-12	5'	GGGaUGGG	CGAUA	UAC	ACAUUG	GUG	AU	CCCACCC	ACAUGAAACCACAG 3'
9-17	5'	GAGGqauGGGA	CCAUAC	GCA	CAU	UGC	UGAA	UCCCCCUC	AAUAGCACCUAC 3'
9-25	5'	GGGAUGGGGA	CCAUA	ACUC	UAAC	GGGU	GAA	UCCCgCAUCUC	GACAAUA 3'
9-26	5'	GGGAuGGG	UGAUA	ACCA	CUC	UGGU	GAA	CCCsUCCC	GACUUGCUCGCA 3'
9-11	5'	GGGaUGGGA	CUAUA	UUCGG	AAU	CUGGA	C	UCCCACCU	GCCUGCCCCCAGA 3'
9-2	5'	GGGAUGGG	CUAUAUA	CAC	GCUG	GUG	AU	CCCAUCUC	AAUUGAAACAACA 3'
9-7	5'	GGAUGGG	CGAUA	ACCA	ACA	UGGU	GAU	CCCAUUC	AUCAUACCCCUACAA 3'
9-28	5'	GGGAuGGGCG	CCAUAC	GCA	CAU	UGC	UGCAU	CGCCUUCCC	GUAAGAAC 3'
9-16	5'	GAGGGaUGGG	CCAUAC	GUGG	ACGA	CUGC	A	CCCGaCCCUU	CAGCCCAGGUC 3'
9-18	5'	GGGaUGGG	CCAUA	ACCA	CUU	UGGU	GAA	CCCACCC	AGCUCCUGUGAUUG 3'
9-14	5'	GGGAuGGGGA	CUAUA	CGU	GAACG	ACU	GCA	UCCaCUUCCC	CGCCAUGG 3'
9-27	5'	GGGaUGGG	UAAUA	ACU	GUA	UGG	UGAA	CCCACCC	AAACUCCCAUGGCUA 3'

Aptamero 9.3t

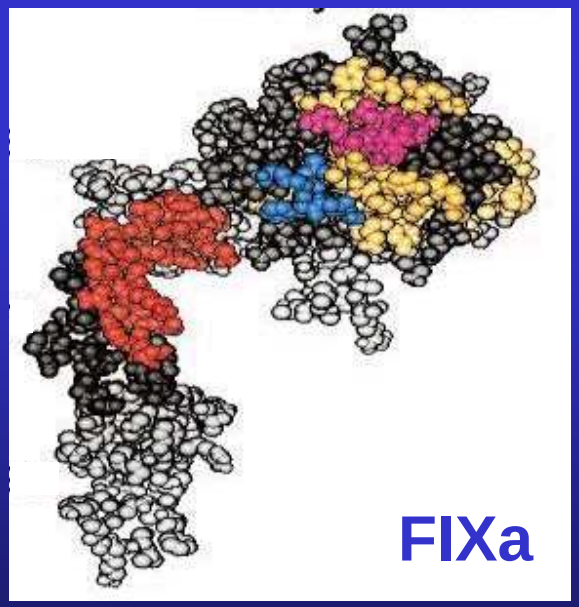
9.3 tM: controllo negativo (aptamero inattivo)



2. SPECIFICITA' per il FIXa

Aptamero
9.3t

5000 volte più specifico



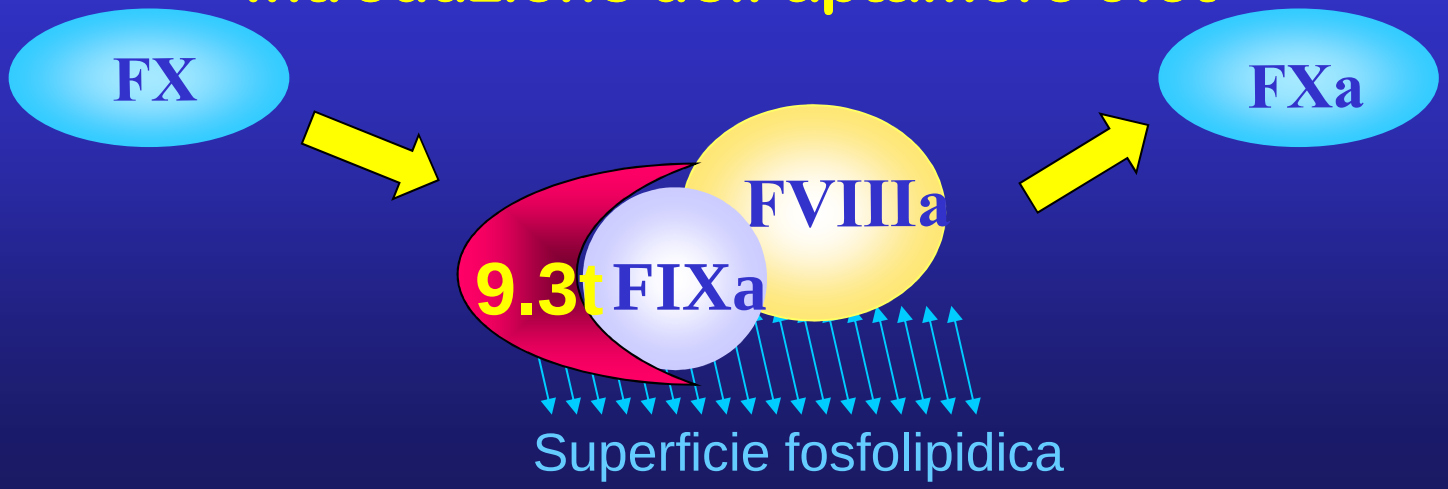
FVIIa **FXa** **FXIa** **APC**

Serin-proteasi

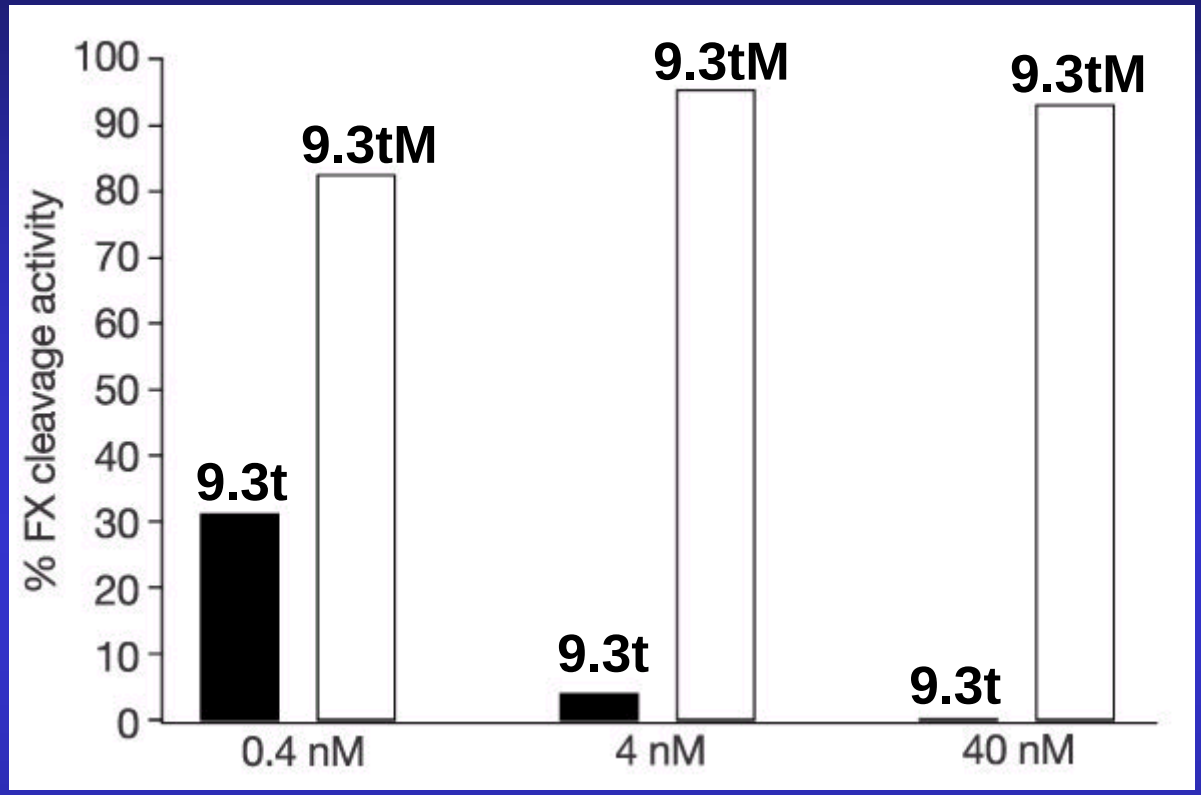
Inibizione IN VITRO dell'attività del FIXa

Assemblamento del complesso

Introduzione dell'aptamero 9.3t



Inibizione IN VITRO dell'attività del FIXa



9.3t: aptamero selezionato
9.3tM: controllo negativo

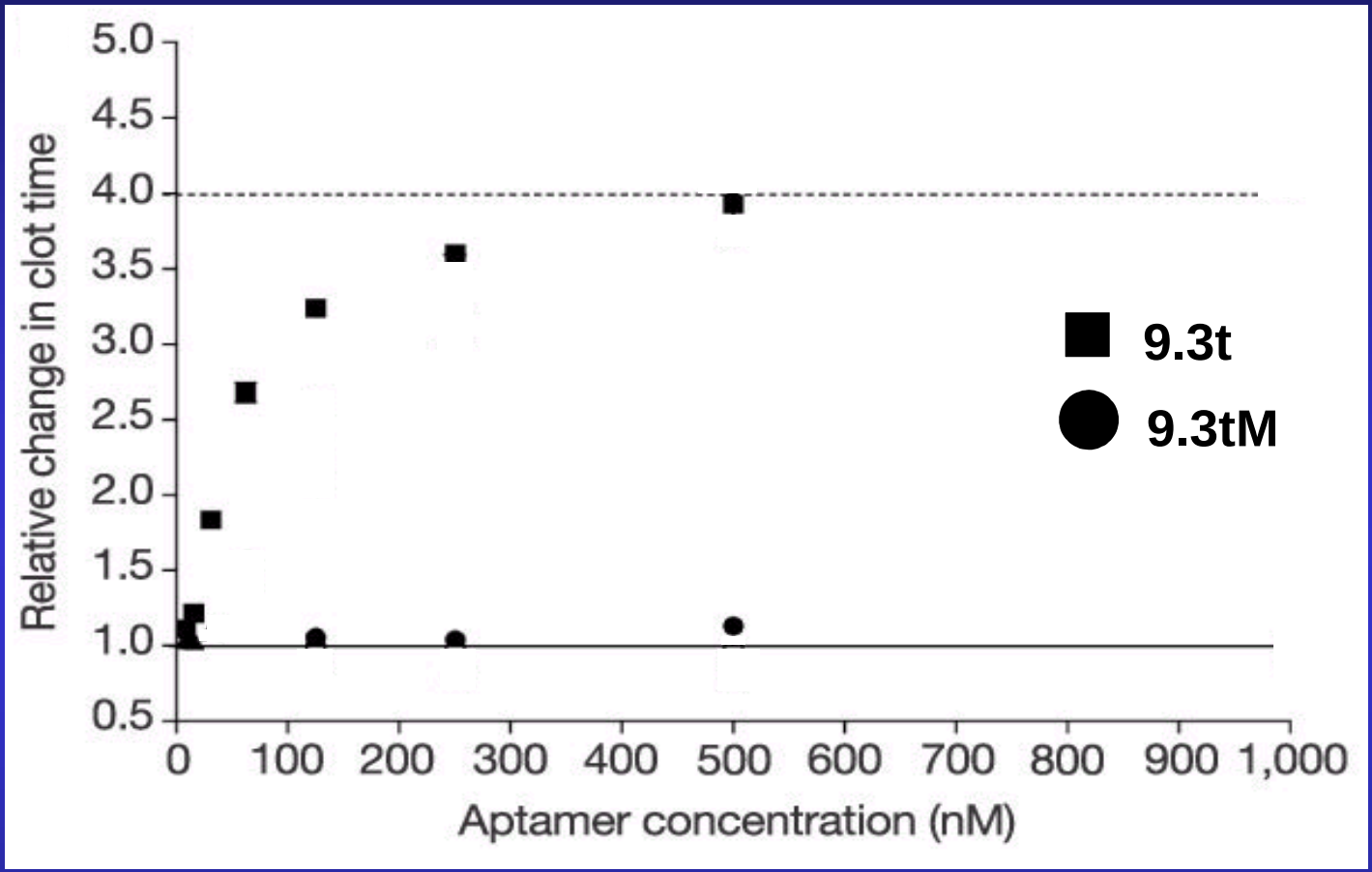
L'aptamero blocca l'attività del FIXa in vitro

Inibizione in plasma umano dell'attività del FIXa

Procedimento:

1. **Aggiunta di diverse concentrazioni di aptamero (9.3t) e controllo negativo (9.3tM) a plasma umano**
2. **Misurazione del tempo di coagulazione del plasma**

Inibizione IN VIVO dell'attività del FIXa

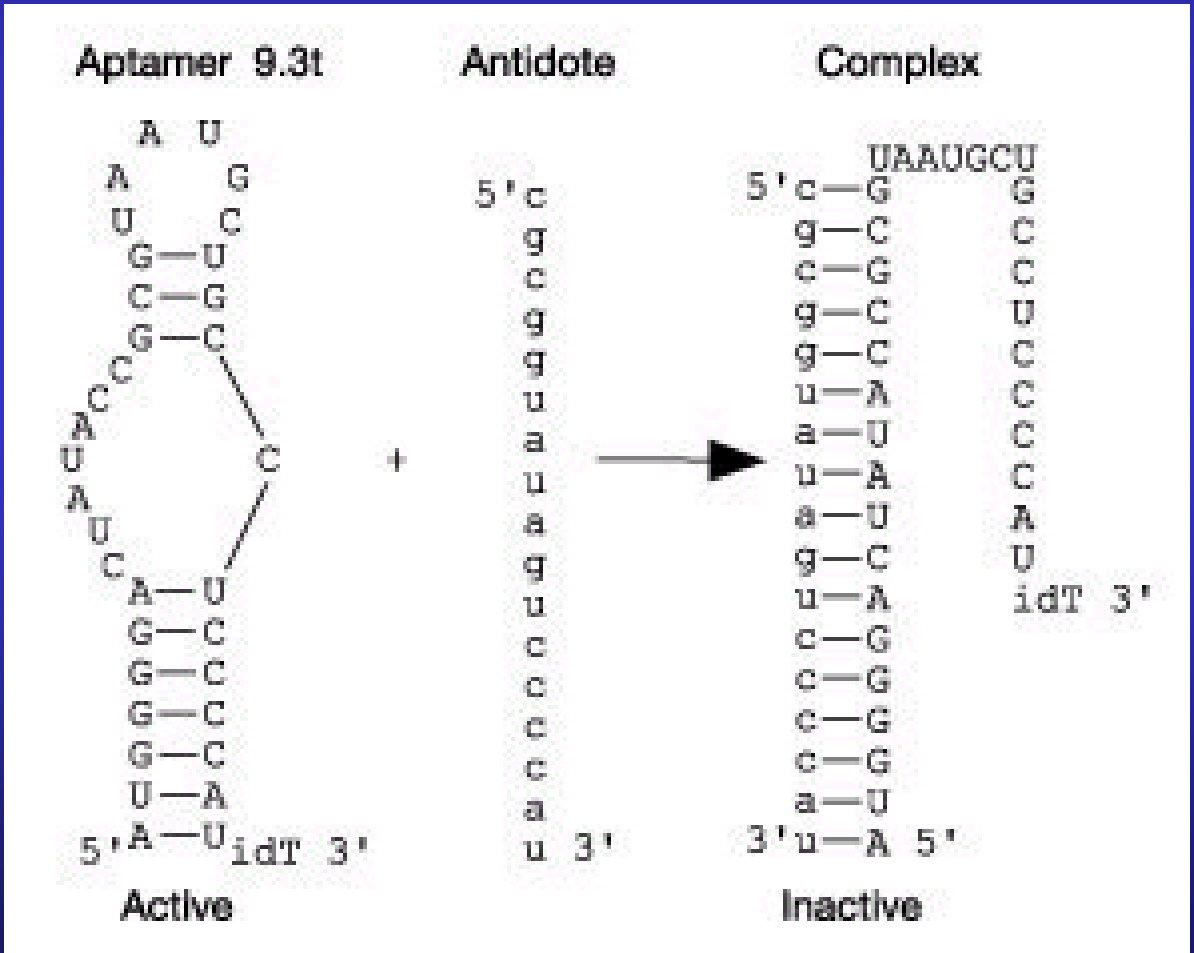


9.3t prolunga il tempo di coagulazione in modo dose-dipendente

L'aptamero inibisce l'attività del FIXa in vivo

Reversibilità dell'azione dell'aptamero: ANTIDOTO

Antidoto = oligo complementare all'aptamero, in grado di alterare la sua conformazione

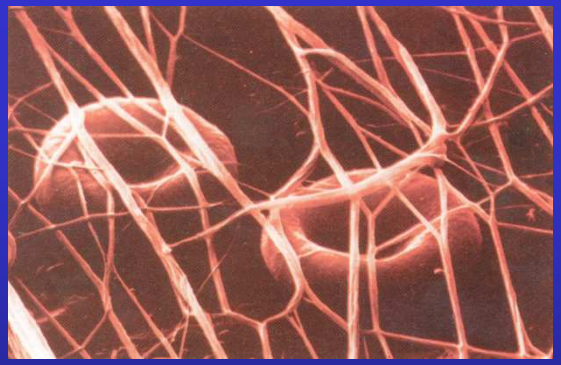


Reversibilità dell'azione dell'aptamero: ANTIDOTO

Plasma + aptamero = inibizione della coagulazione

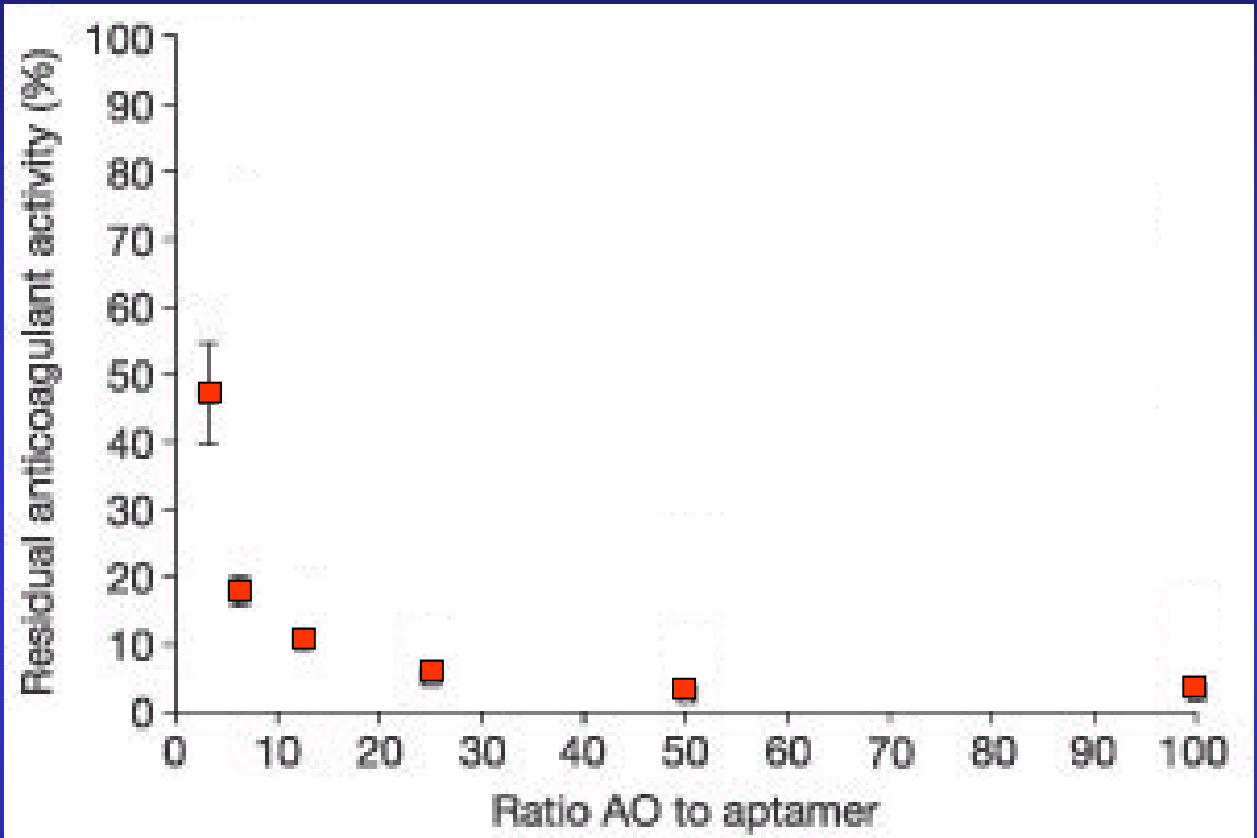
↓

Plasma non coagulato + antidoto = coagulazione



Si misura la capacità del plasma di coagulare entro 10 min.

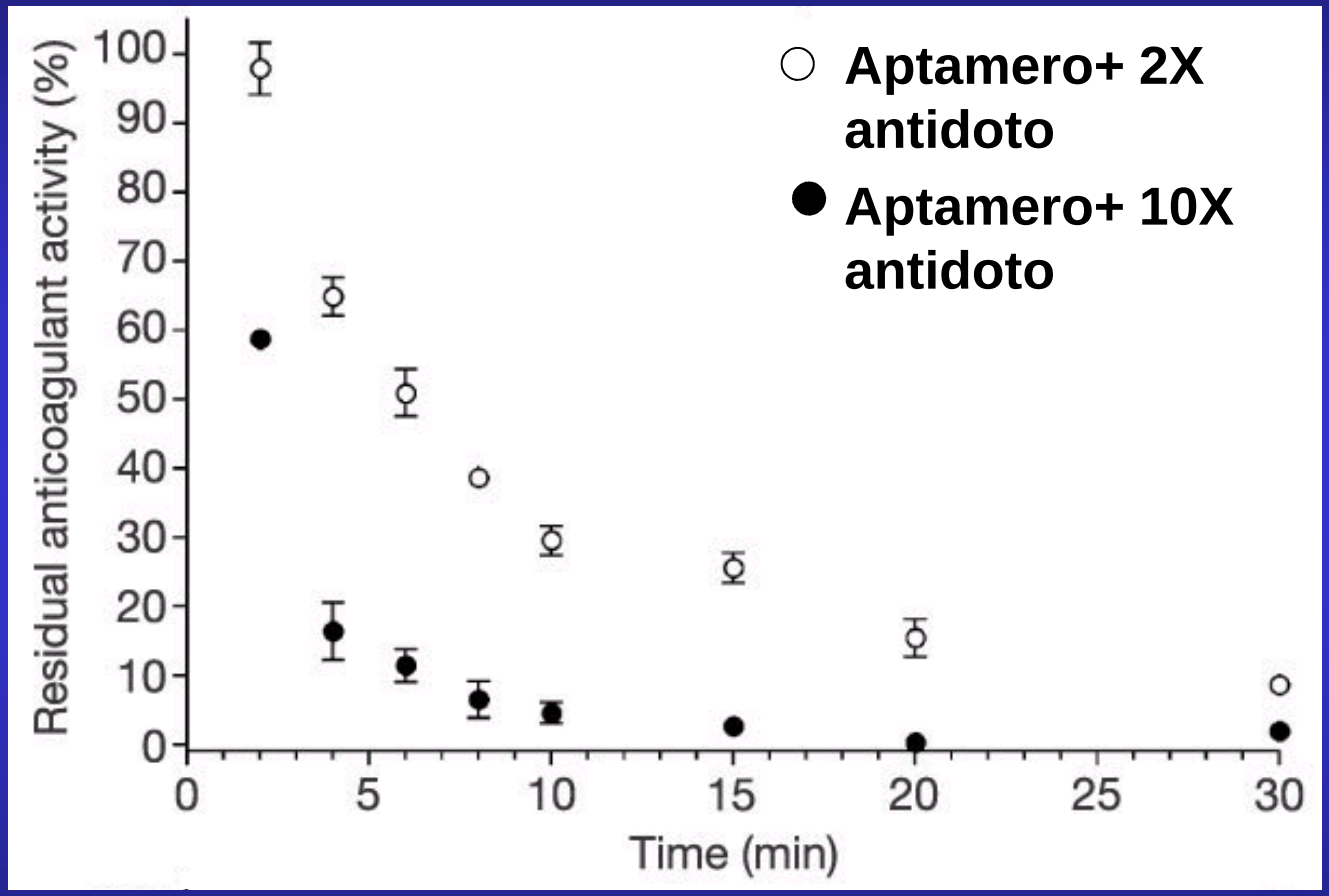
Reversibilità dell'azione dell'aptamero: ANTIDOTO



Quantità crescenti di antidoto / aptamero 9.3t (50 nM)

L'antidoto neutralizza l'azione dell'aptamero

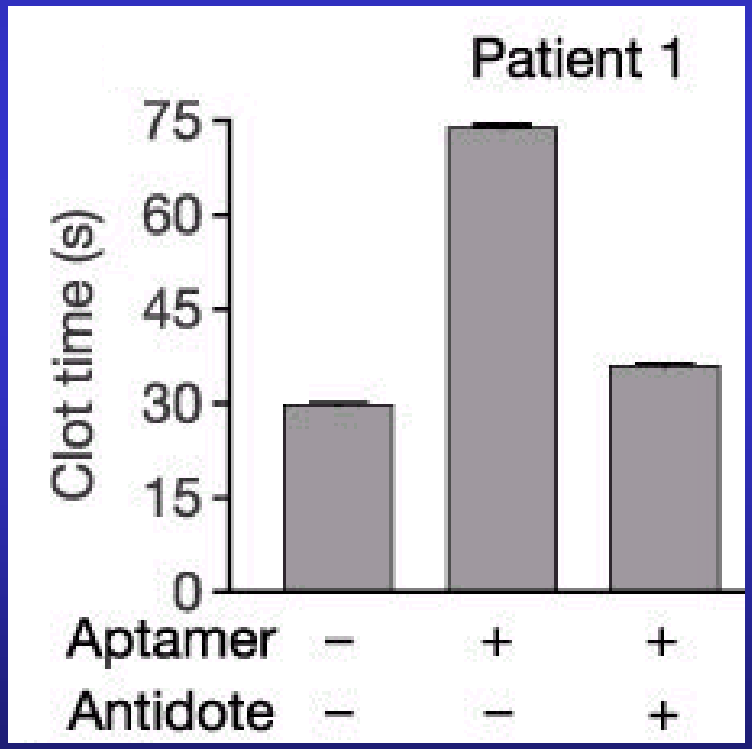
Reversibilità dell'azione dell'aptamero: ANTIDOTO



L'azione dell'antidoto è rapida e dose-dipendente

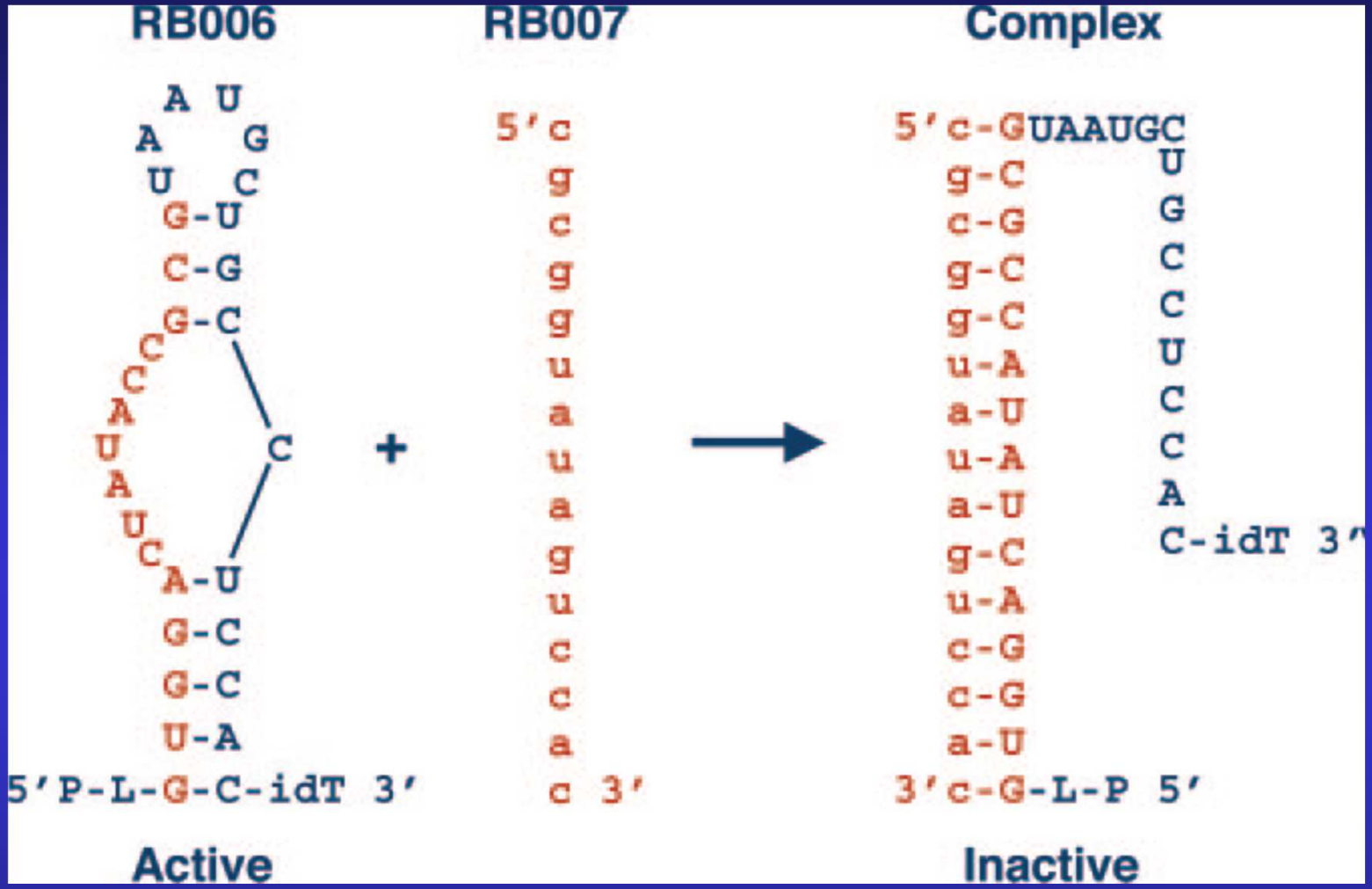
Efficacia di aptamero e antidoto su pazienti

6 Pazienti con trombosi pazienti non sottoponibili ai normali trattamenti anticoagulanti

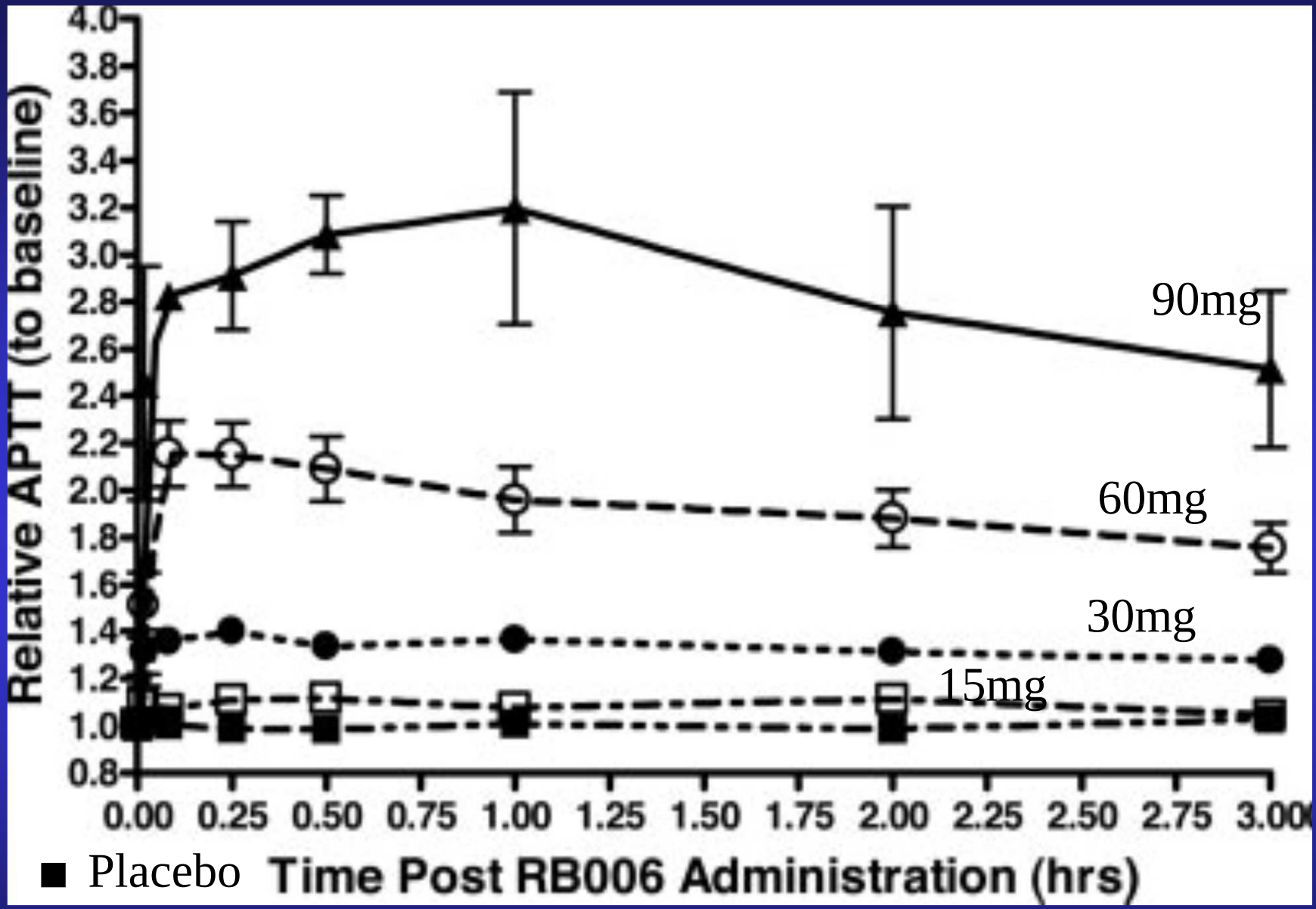


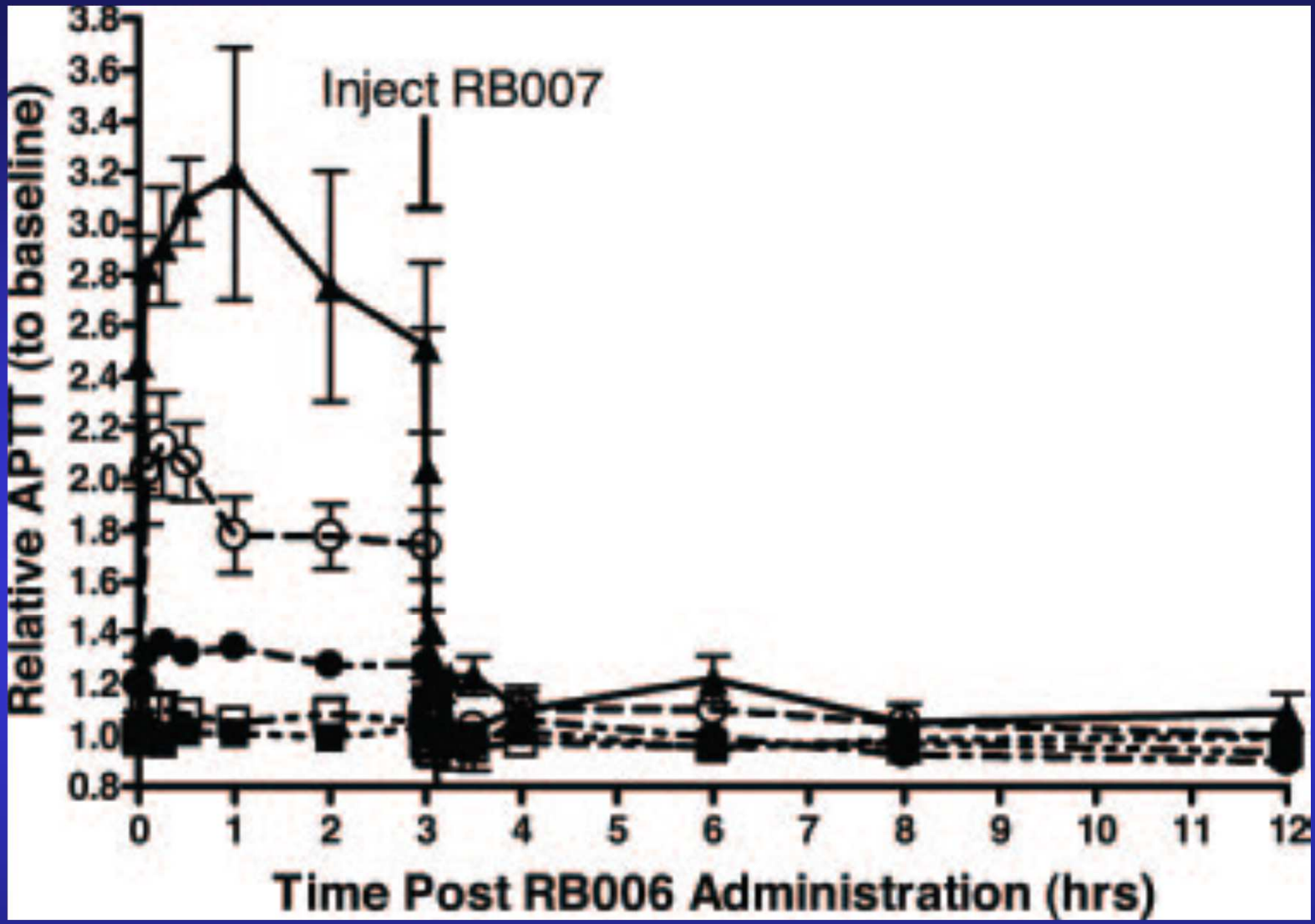
First-in-human experience of an antidote-controlled anticoagulant using RNA aptamer technology: a phase 1a pharmacodynamic evaluation of a drug-antidote pair for the controlled regulation of factor IXa activity.

- Selectivity, titratability, rapidity of onset, and active reversibility are desirable pharmacological properties of anticoagulant therapy administered for acute indications and collectively represent an attractive platform to maximize patient safety. A novel anticoagulation system (REG1, Regado Biosciences), developed using a protein-binding oligonucleotide to factor IXa (drug, RB006) and its complementary oligonucleotide antidote (RB007), was evaluated in healthy volunteers. The primary objective was to determine the safety profile and to characterize the pharmacodynamic responses in this first-in-human study. METHODS AND RESULTS: Regado 1a was a subject-blinded, dose-escalation, placebo-controlled study that randomized 85 healthy volunteers to receive a bolus of drug or placebo followed 3 hours later by a bolus of antidote or placebo. Pharmacodynamic samples were collected serially. Subject characteristics were the following: median age, 32 years (interquartile range, 23 to 39 years); female gender, 35%; and median weight, 79 kg (interquartile range, 70 to 87 kg). No significant differences were found in median hemoglobin, platelet, creatinine, or liver function studies. There were no significant bleeding signals associated with RB006, and overall, both drug and antidote were well tolerated. One serious adverse event, an episode of transient encephalopathy, occurred in a subject receiving the low intermediate dose of RB006. The subject's symptoms resolved rapidly, and no further sequelae occurred. A predictable dose-pharmacodynamic response, reflected in activated partial thromboplastin time measurements, was seen after administration of the bolus of drug, with a clear correlation between the peak posttreatment activated partial thromboplastin time and post hoc weight-adjusted dose of drug (correlation coefficient, 0.725; $P<0.001$). In subjects treated with drug, antidote administration reversed the pharmacological activity of the drug, with a rapid (mean time, 1 to 5 minutes across all dose levels) and sustained return of activated partial thromboplastin time to within the normal range. The activated clotting time followed a similar anticoagulant response and reversal pattern. As anticipated, prothrombin time remained unchanged compared with baseline. CONCLUSIONS: These observations represent a first-in-human experience of an RNA aptamer and its complementary oligonucleotide antidote used as an anticoagulant system. The findings contribute to an emerging platform of selective, actively reversible anticoagulant drugs for use among patients with thrombotic disorders of the venous and arterial circulations.



P polyethylene glycol; idT, inverted deoxythymidine


Relative APTT (to baseline)
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.00 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00 2.25 2.50 2.75 3.00
Time Post RB006 Administration (hrs)
90mg
60mg
30mg
15mg
Placebo


Inject RB007
Relative APTT (to baseline)
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
1
2
3
4
5
6
7
8
9
10
11
12
Time Post RB006 Administration (hrs)

Pharmacodynamic effects of RB006 at 0 to 3 hours after RB006 administration.

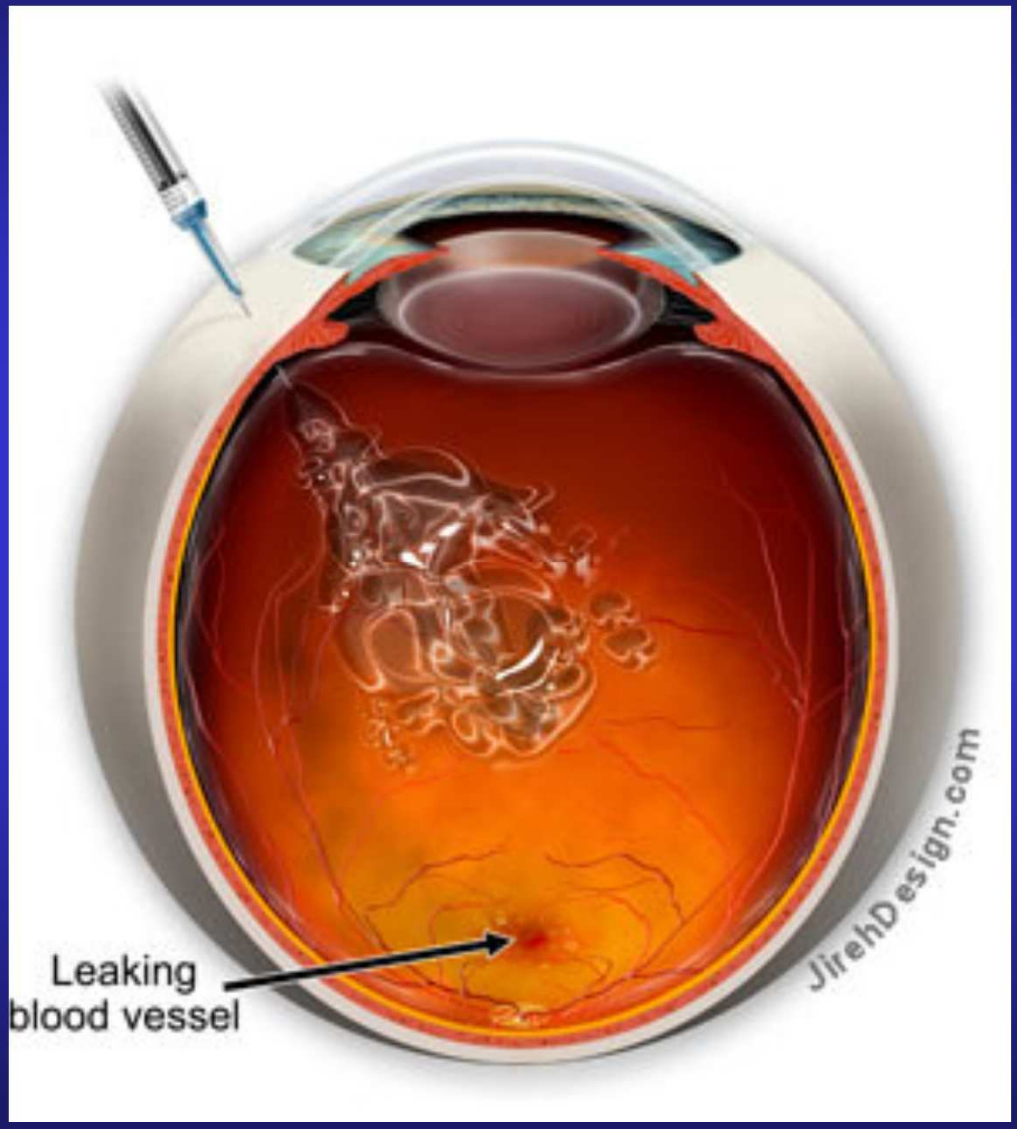
The relative increase in APTT over baseline for each subject receiving RB006 before RB007 or placebo administration (all subjects assigned to arms 2 and 3) is shown vs subjects receiving placebo. Data represent the mean SEM for all subjects receiving treatment at each dose level.

CONCLUSIONI

1. Aptameri contro il FIXa sono potenti anticoagulanti

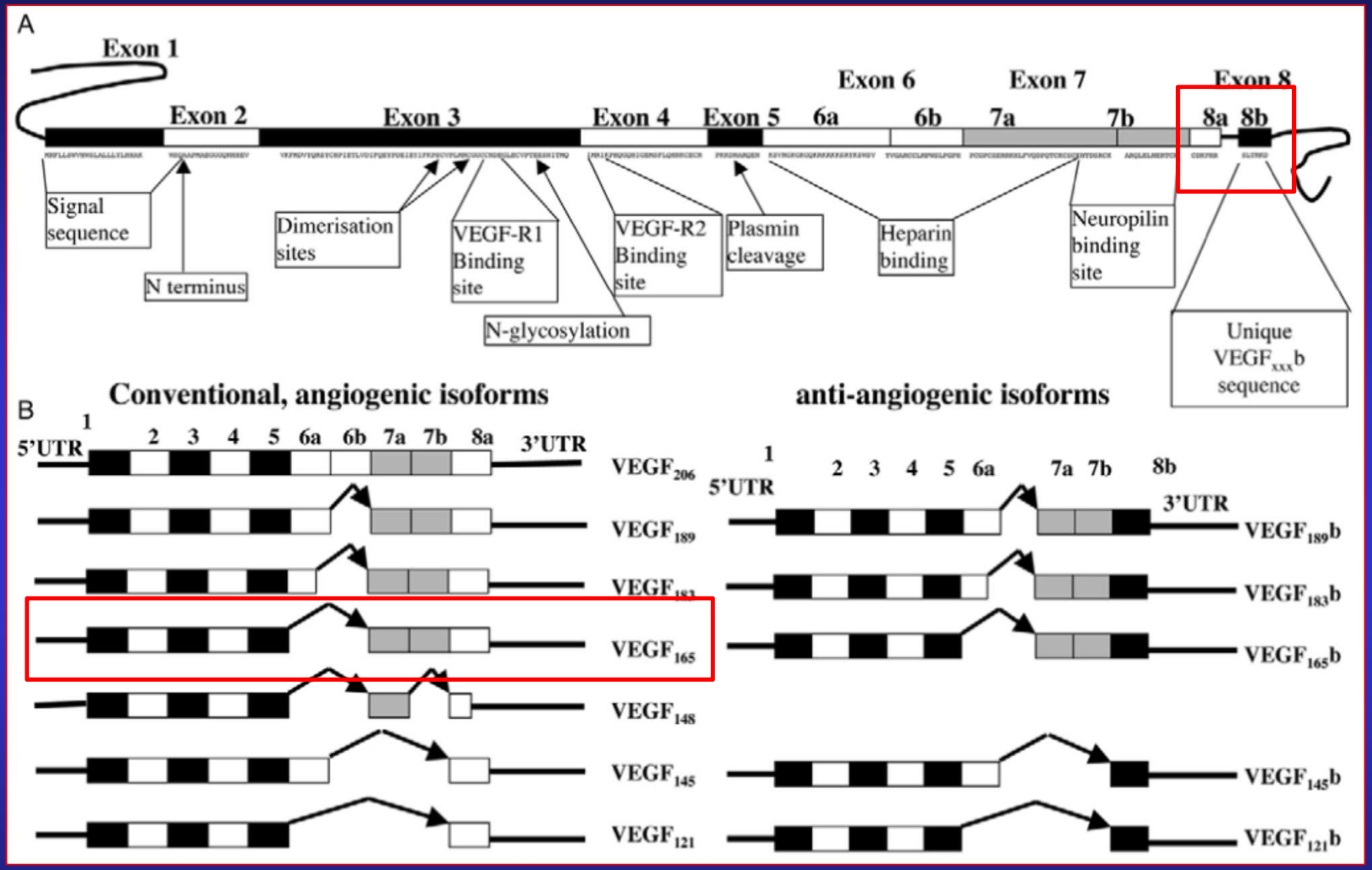
2. Oligonucleotidi complementari agli aptameri possono agire da antidoti e neutralizzare l'azione anticoagulante

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


Leaking
blood vessel
JirehDesign.com

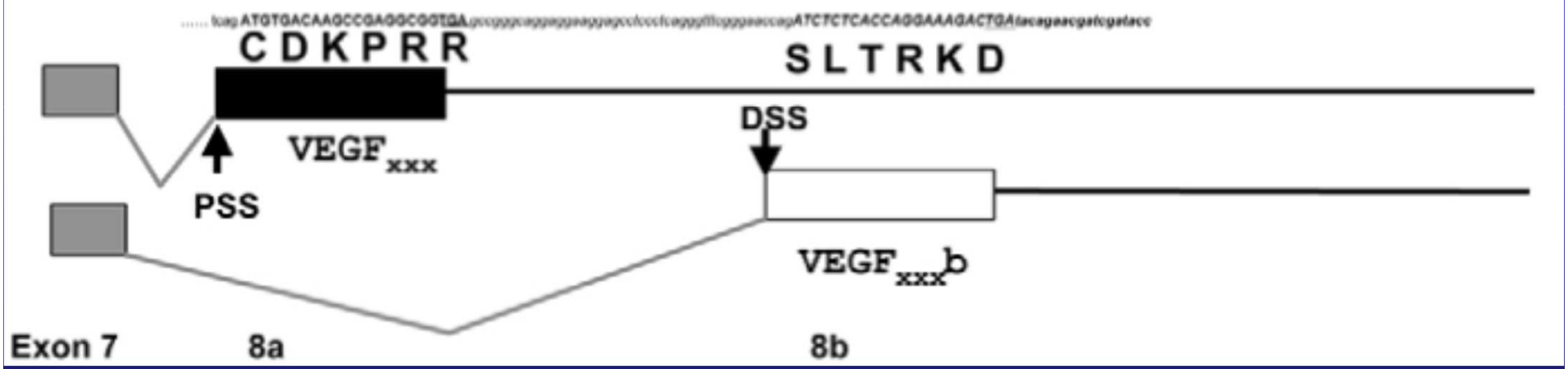
VEGF and Macula Degeneration

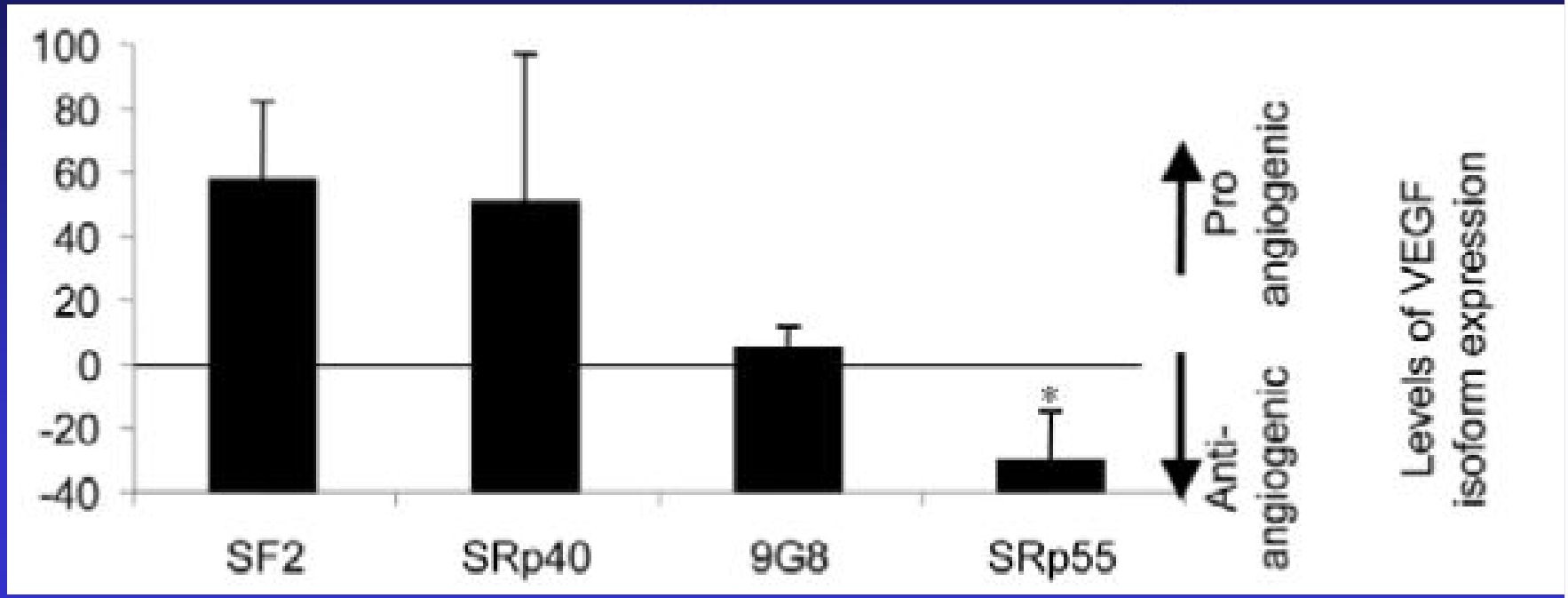
- Both clinical and preclinical findings have implicated vascular endothelial growth factor (VEGF) in the pathophysiology macular edema and degeneration.
- *VEGF is both a potent enhancer of vascular permeability and a key inducer of angiogenesis.
- *VEGF levels are elevated in the eyes of patients.
- Injection of VEGF (the VEGF165 isoform in particular) into healthy eyes of animals can induce associated ocular pathologies


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
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.

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

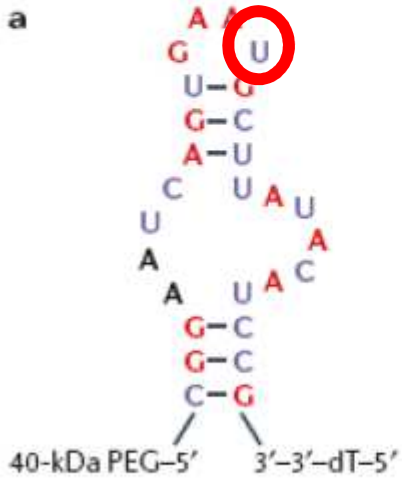
Starita C, Patel M, Katz B, Adamis A

- Pegaptanib, a novel RNA aptamer currently used in the treatment of agerelated macular degeneration, binds and inactivates VEGF165 and has been shown in animal models to reverse the blood-retinal barrier breakdown.

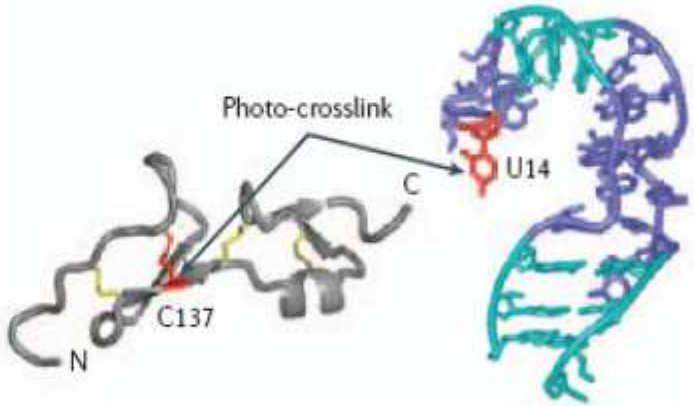
Il pegaptanib e` un antagonista selettivo del $VEGF_{165}$

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**)

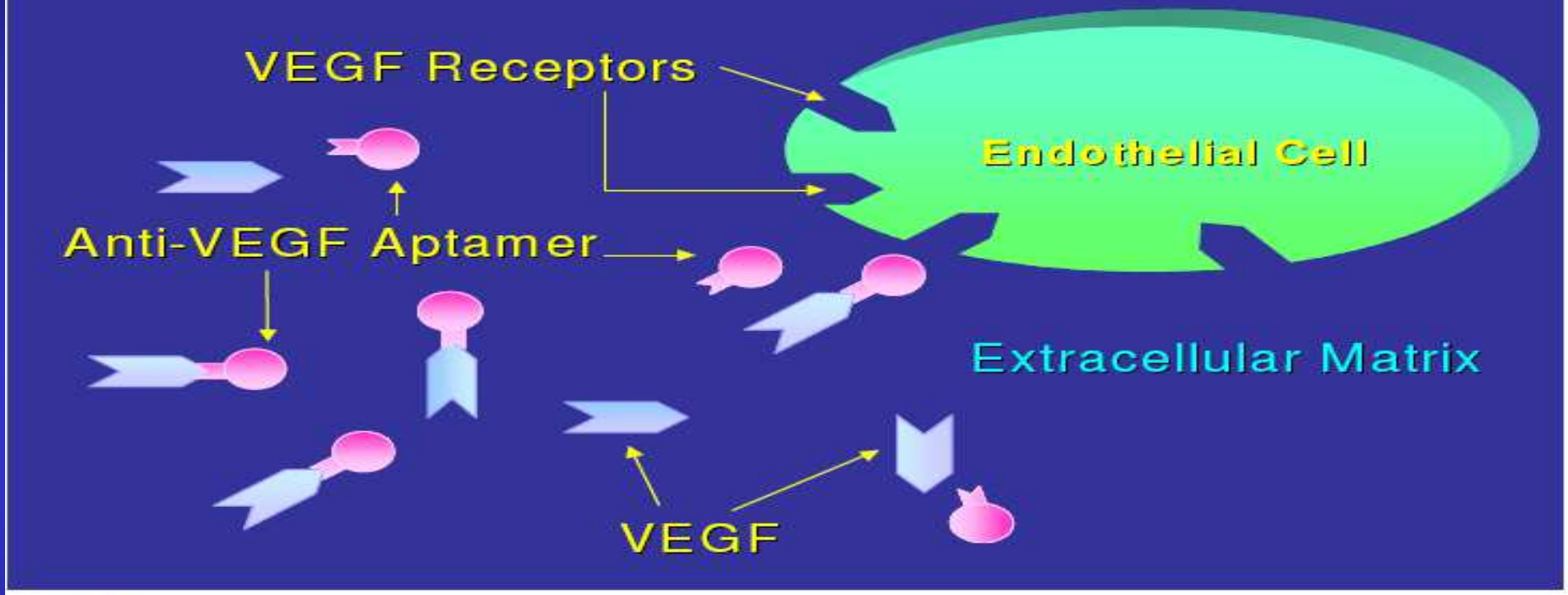


a | Sequenza e struttura secondaria del pegaptanib.



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

Extracellular Neutralization of VEGF



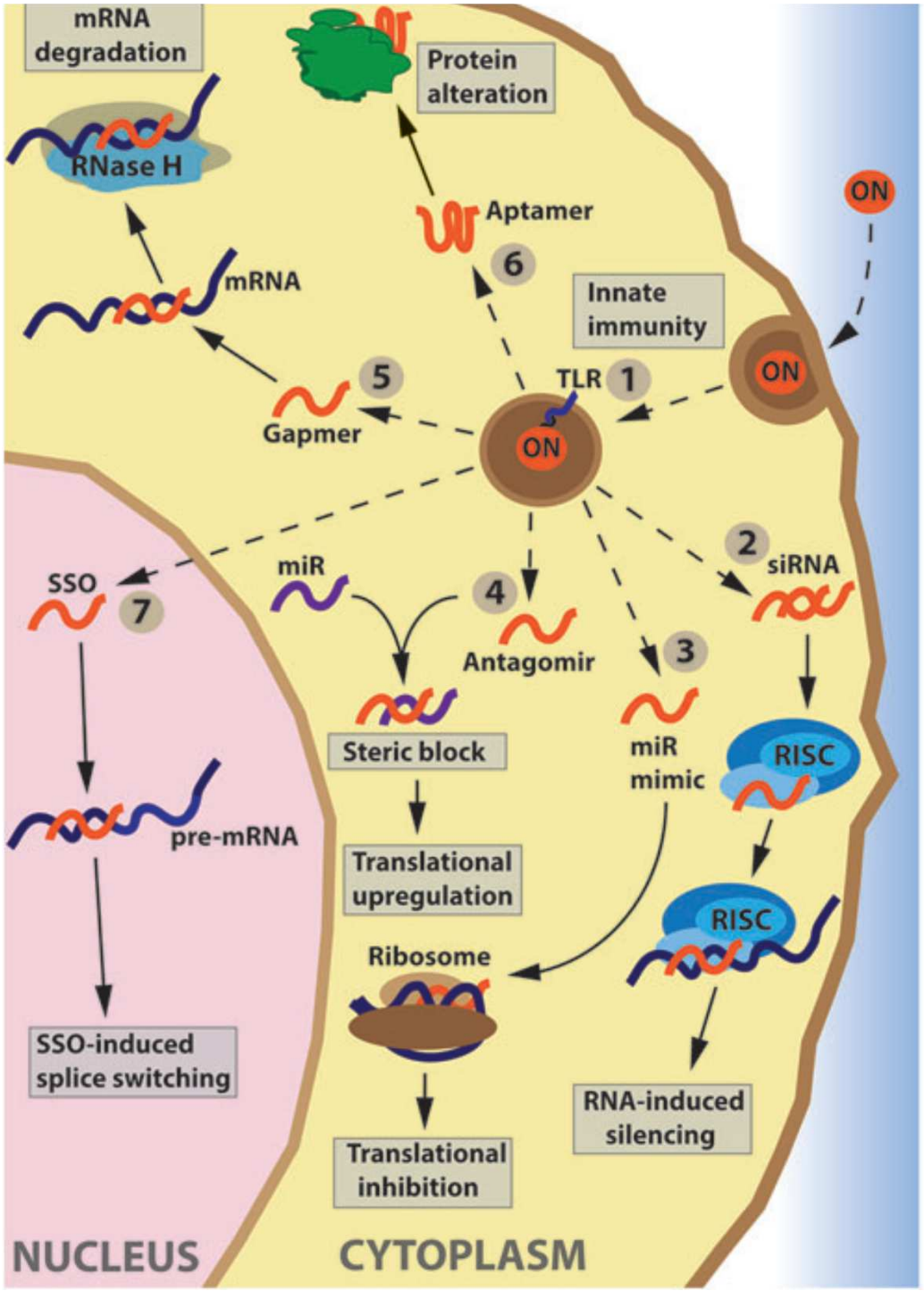
- Il Pegaptanib si lega specificamente al VEGF-165, impedendone l'aggancio con il suo recettore

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

V.I.S.I.O.N. Clinical Trial Group. *Retina* 2005;25:815-827.

- Gli Aptameri fanno parte di un gruppo ampio di metodologie basate su oligonucleotidi



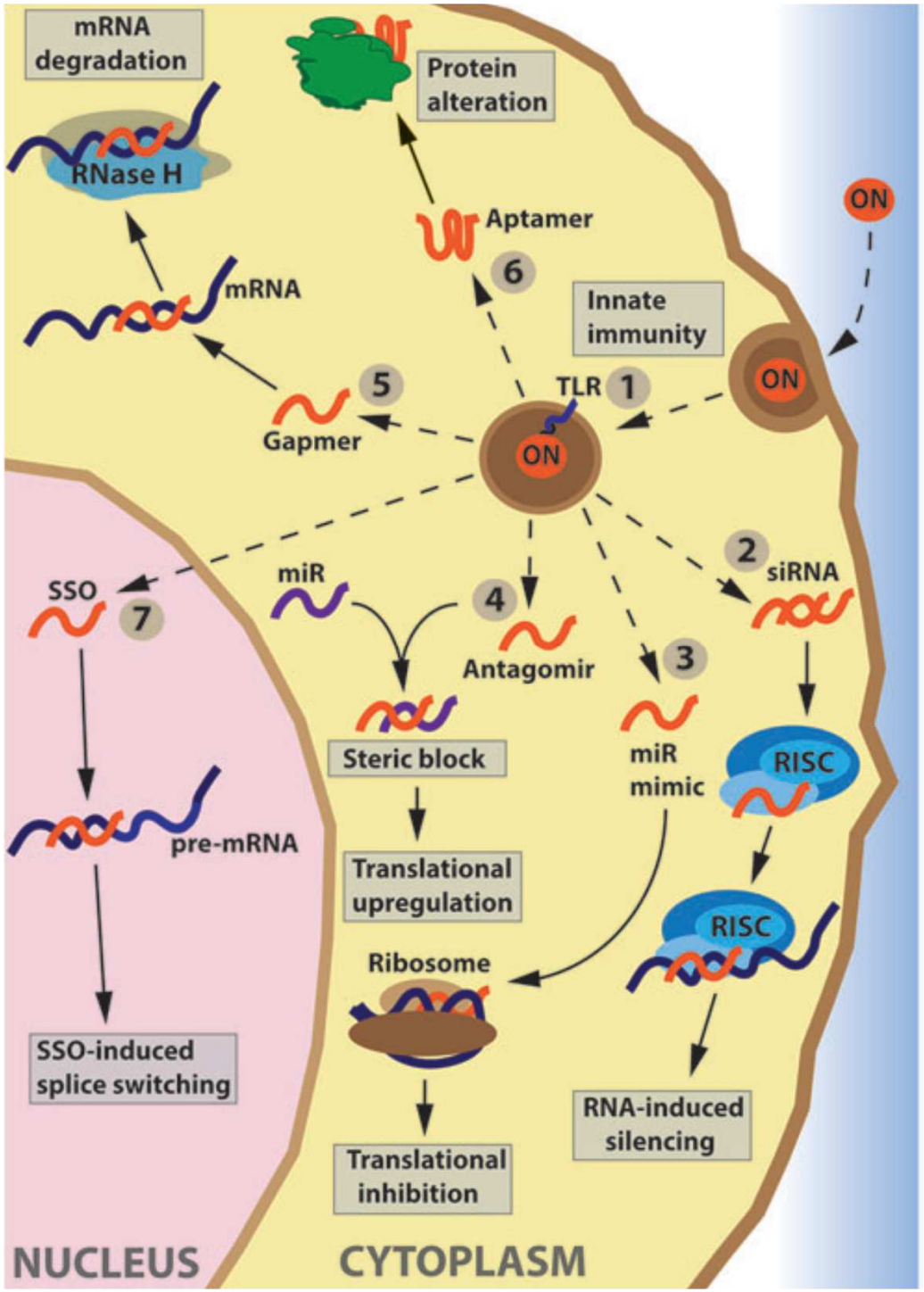
Mechanisms for Oligonucleotide use in the clinic.

(1) Binding to Toll-like receptors
(TLRs) in the endosome.

(2) Small interfering RNA (siRNA).

(3) Micro-RNA (miR) mimic.

(4) Antagomir, sterically blocking endogenous miR.



(5) Gapmer AON, inducing RNase H degradation (steric block ONs also exist).

(6) Aptamer, binding alters protein surface.

(7) Splice switching ON (SSO).
Not
depicted are anti-gene ONs, and ONs directed against nuclear regulatory RNA species, which are not yet used clinically