THE FRITSMA FACTOR

Your Interactive Hemostasis Resource

Aspirin: The 1899 Wonder Drug

Monitoring Aspirin's Antiplatelet Property

George A Fritsma MS, MLS
The Fritsma Factor, Your interactive Hemostasis Resource
Precision BioLogic Inc, Dartmouth, Nova Scotia
www.fritsmafactor.com

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Aspirin Therapy; The Participant…

- Diagrams the biochemical pathways of aspirin's anti-platelet and anti-inflammatory effects.
- Employs aspirin to reduce risk of cardiovascular disease.
- Reviews effect of aspirin on platelet activation in inflammatory disease, cancer, and depression.
- Orders aspirin assays for compliance, efficacy, and dosage.

Please Silence Your Phone

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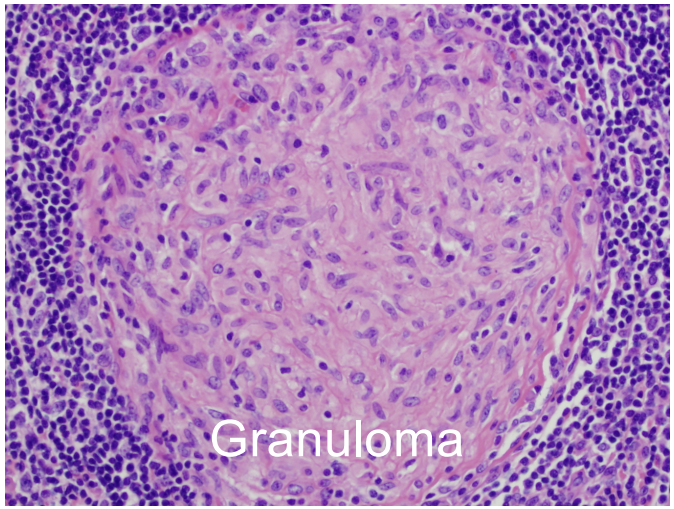
Systemic Sarcoidosis Symptoms

- Chronic inflammation with granulomas in lungs, spleen, throughout body
  - Persistent intense dry cough, hoarseness
  - Tender reddish bumps or patches on the skin
  - Red and teary eyes or blurred vision
  - Swollen and painful joints
  - Enlarged lymph glands in neck, armpits, groin
  - Enlarged lymph glands in chest and around lungs
- Cardiac sarcoid--fatal
- Rx: low-dose prednisone, plaquenil, azathioprine, tumor necrosis factor



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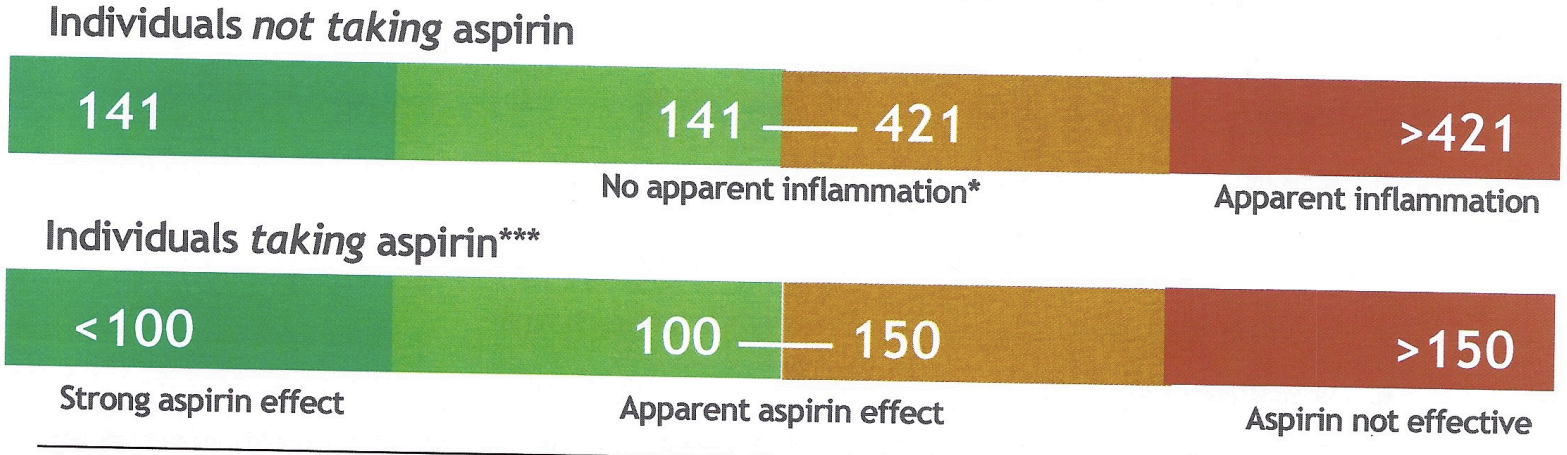
Chronic Inflammation Test

Date of Testing: 06/08/18
Date of Collection: 05/25/18
Healthcare Practitioner: N/A

Test Name	Test Score*
Chronic Inflammation Test Urinary 11-dehydrothomboxane B_2	993



*Test score is calculated by dividing pg 11-dehydrothromboane B_2/mg creatinine by 10
*Test score valid for individuals 18 years of age and older

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Chronic Inflammation Test with Aspirin

Collected	ASA mg/day	Score	RI
5/25/18	0	993	<421
5/29/18	81	134	<150
12/28/18		163	
1/8/19		158	
2/1/19		131	

Aspirin resistant

Have we resolved the inflammation or just suppressed the platelets?

Inflammatory Markers

- Proinflammatory cytokines
  - Tumor necrosis factor α
  - Interleukin 1 β
  - Interleukin 6
  - Interferon γ
- Prostaglandins and leukotrienes
  - Thromboxane A_2
  - Thromboxane B_2
  - 11-dehydro thromboxane B_2
- Acute phase reactants: CRP, VWF, factor VIII, prothrombin, fibrinogen, complement factors, ferritin, ceruloplasmin, serum amyloid A and haptoglobin
- Hundreds more

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Felix Hoffman; 1897

- "Willow-bark Salix" appears in 1534 BC Egyptian papyri
- 1800s: Spirea (Meadowsweet) leaves
  - Salicylic acid—required large quantities and caused gastric pain
- 8/10/1897: Felix Hoffman synthesized pure, stable acetyl salicylic acid at Bayer Labs in Leverkusen, Germany
  - Aspirin: a = acetyl; spir = Spirea
- 1899: Bayer lab mixes ASA with starch: made the first tablet
- No scrip: 5 grains [~325 mg], WHO essential medicine list
- 2017: 40,000 tons of aspirin produced, 50,000,000 people
- Uruguayan stamp shows Hoffman, a willow branch, and his signature from the Bayer lab record.

Mann CC, Plummer ML. The Aspirin Wars: Money, Medicine, and 100 Years of Rampant Competition. New York: Knopf 1991.

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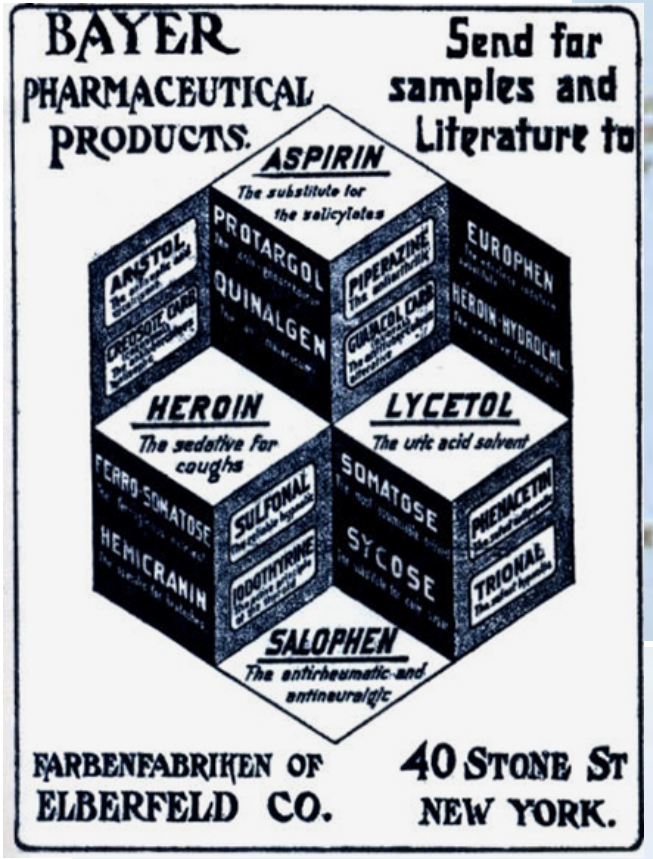
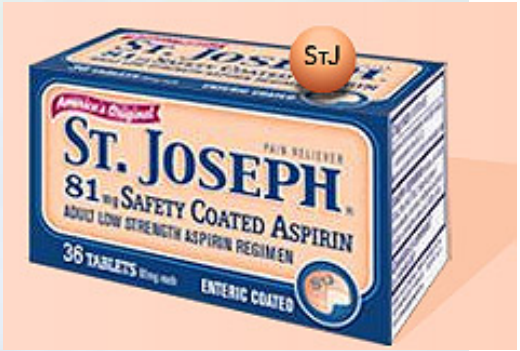


1910 Ad

EL CORREO



ASPIRINA

Bayer

Dr. Lawrence Craven: 1948

- California PCP documented 400 men on aspirin had no MIs from 1948–50.
  - Recorded Aspergum related to post-T&A bleeding
  - Extended studies to 8000 men
  - Recommended an aspirin a day to reduce risk of heart attacks, was largely ignored
  - Died of a heart attack at age 74
- 1971: JB Smith demonstrated aspirin's inhibition of prostaglandin synthesis

Craven LL. Acetylsalicylic acid, possible preventive of coronary thrombosis. Ann Western Med 1950;4: 95–9.
Vane JN. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 1971;231:232–5.
Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nature 1971; 231: 235–7.

BAYER Pharmaceutical Products

HEROIN—HYDROCHLORIDE

is pre-eminently adapted for the manufacture of cough elixirs, cough balsams, cough drops, cough lozenges, and cough medicines of any kind. Price in 1 oz. packages, $4.85 per ounce; less in larger quantities. The efficient dose being very small (1-48 to 1-24 gr.), it is

The Cheapest Specific for the Relief of Coughs

(In bronchitis, phthisis, whooping cough, etc., etc.)

WRITE FOR LITERATURE TO

FARBENFABRIKEN OF ELBERFELD COMPANY

SELLING AGENTS

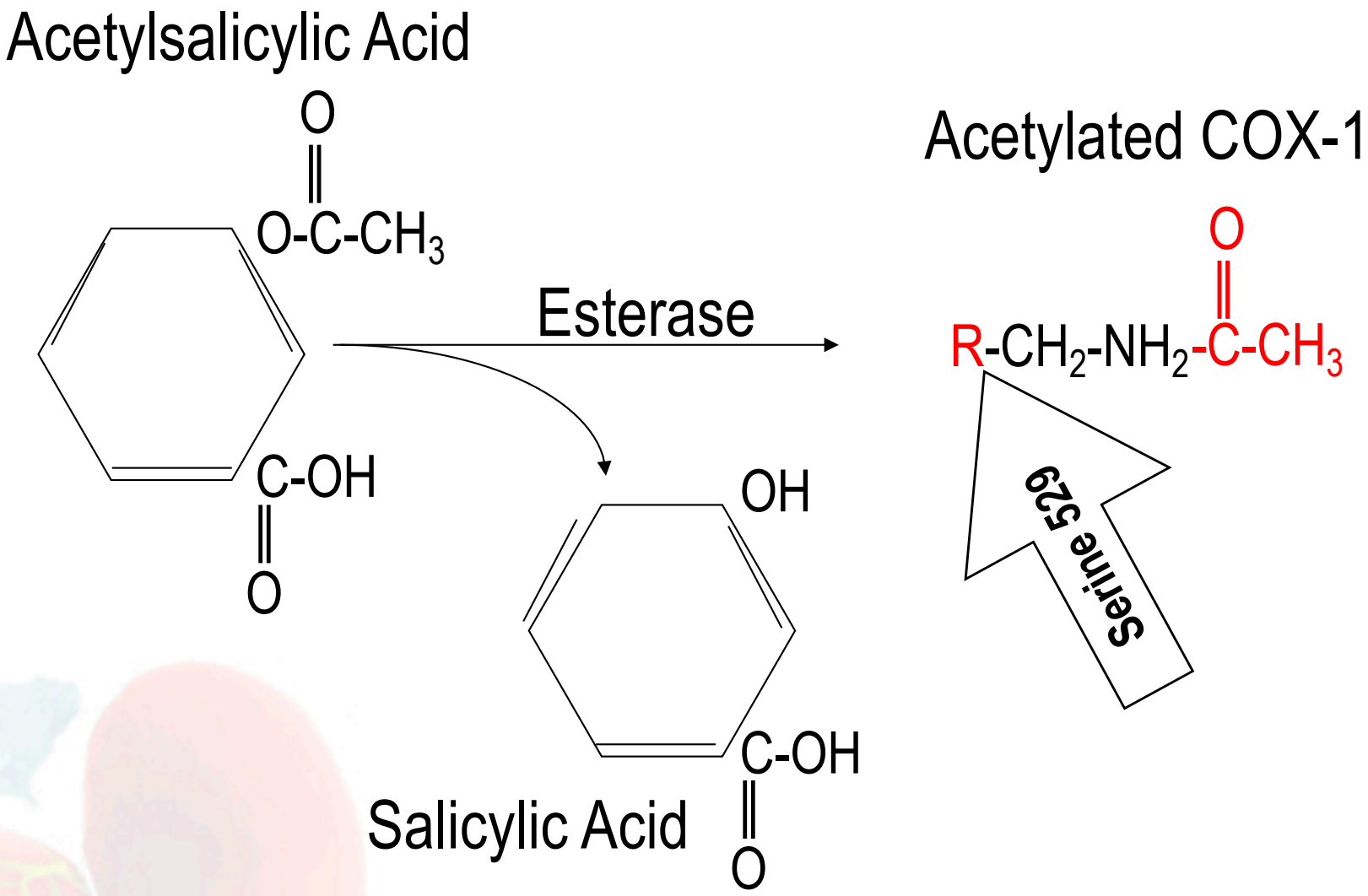
P. O. Box 2160 40 Stone Street, NEW YORK

Irreversible Acetylation of Cyclooxygenase-1

- PLT dense tubular system COX-1 acetylated at ser_{529}
  - Blocks arachidonic acid's access to reactive "tunnel"
  - Active site amino acid tyr_{385} unmodified by acetyl group but blocked
- Platelet loses COX-1 activation pathway
  - AKA *eicosanoid synthesis* pathway or *prostaglandin* pathway
  - Total function recovery ~10%/day as new platelets are produced
- Adhesion and shear-induced aggregation remain
  - VWF-dependent

Vane JR, Botting RM. Mechanism of action of aspirin-like drugs. Semin Arthr Rheum 1997; 25 Suppl I: 2-10.

Acetylation of Cyclooxygenase-1



Pedersen AK, Fitzgerald GA. Dose-related kinetics of aspirin: Presystemic acetylation of platelet cyclooxygenase. New Engl J Med 1984; 311: 1206-11.

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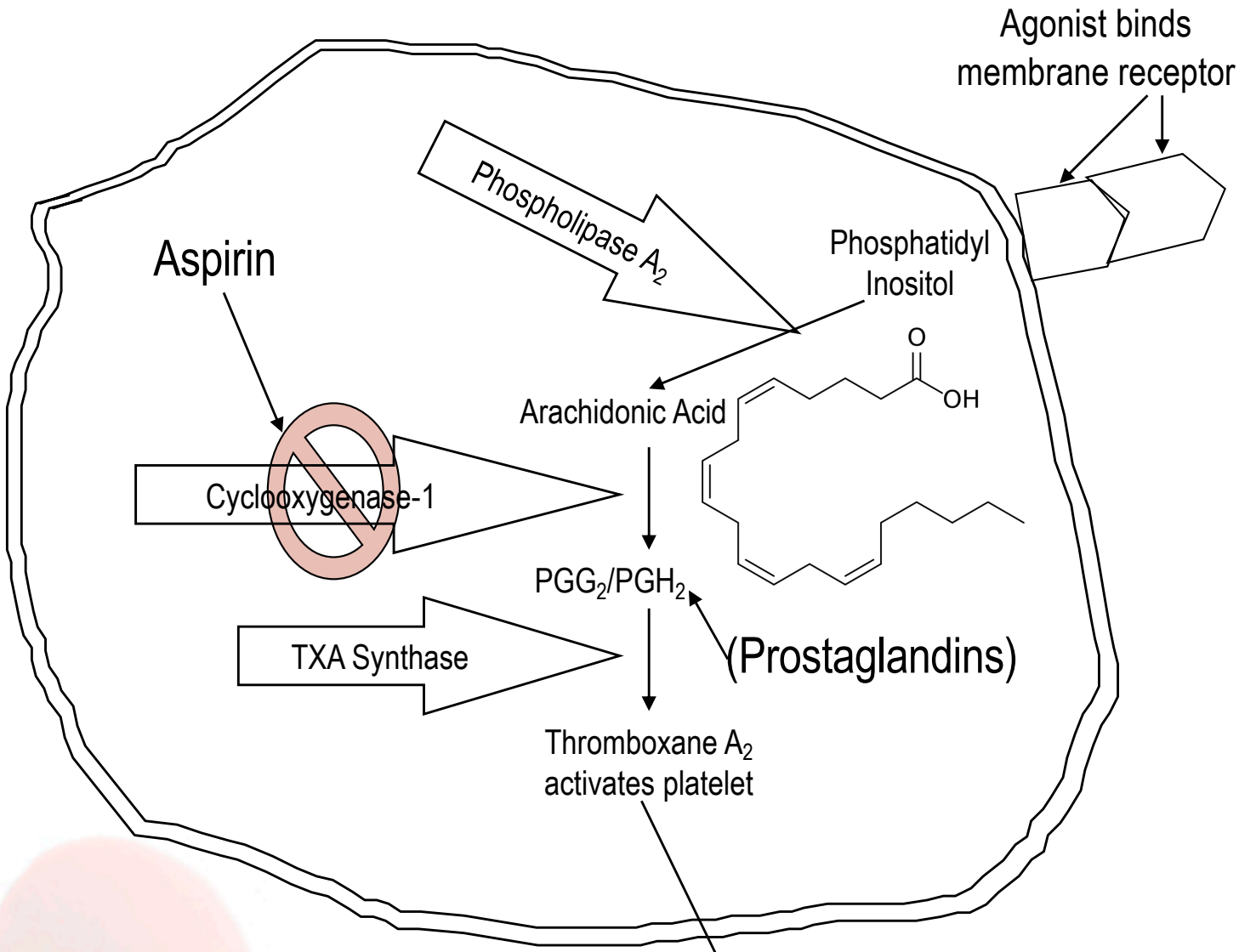
Aspirin acetylates COX-1 and reduces TXA_2 production



Thromboxane B_2 is a stable, measurable plasma product that is reduced in ASA therapy, converts to water-soluble urinary 11-dehydro TXB_2

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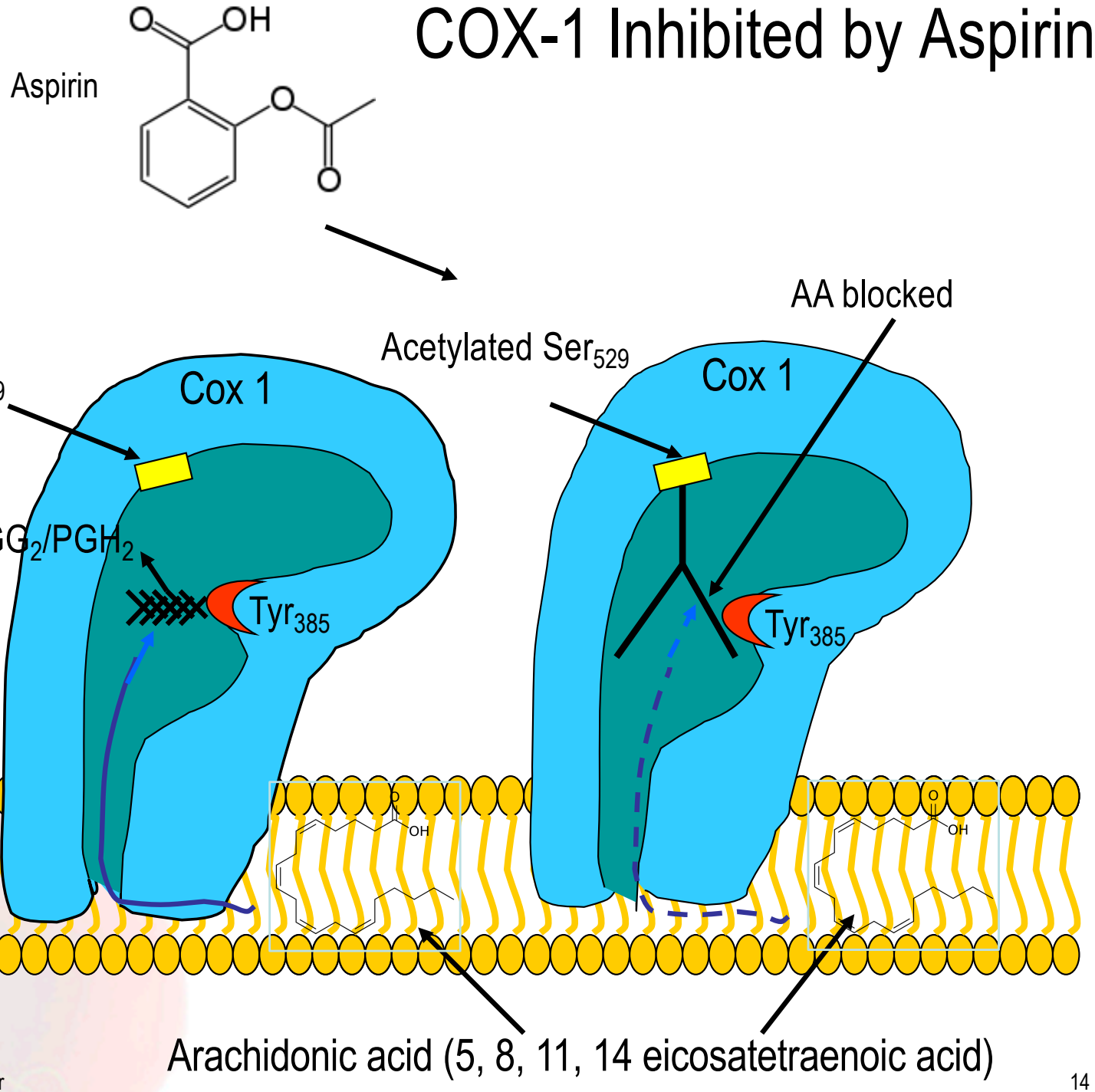
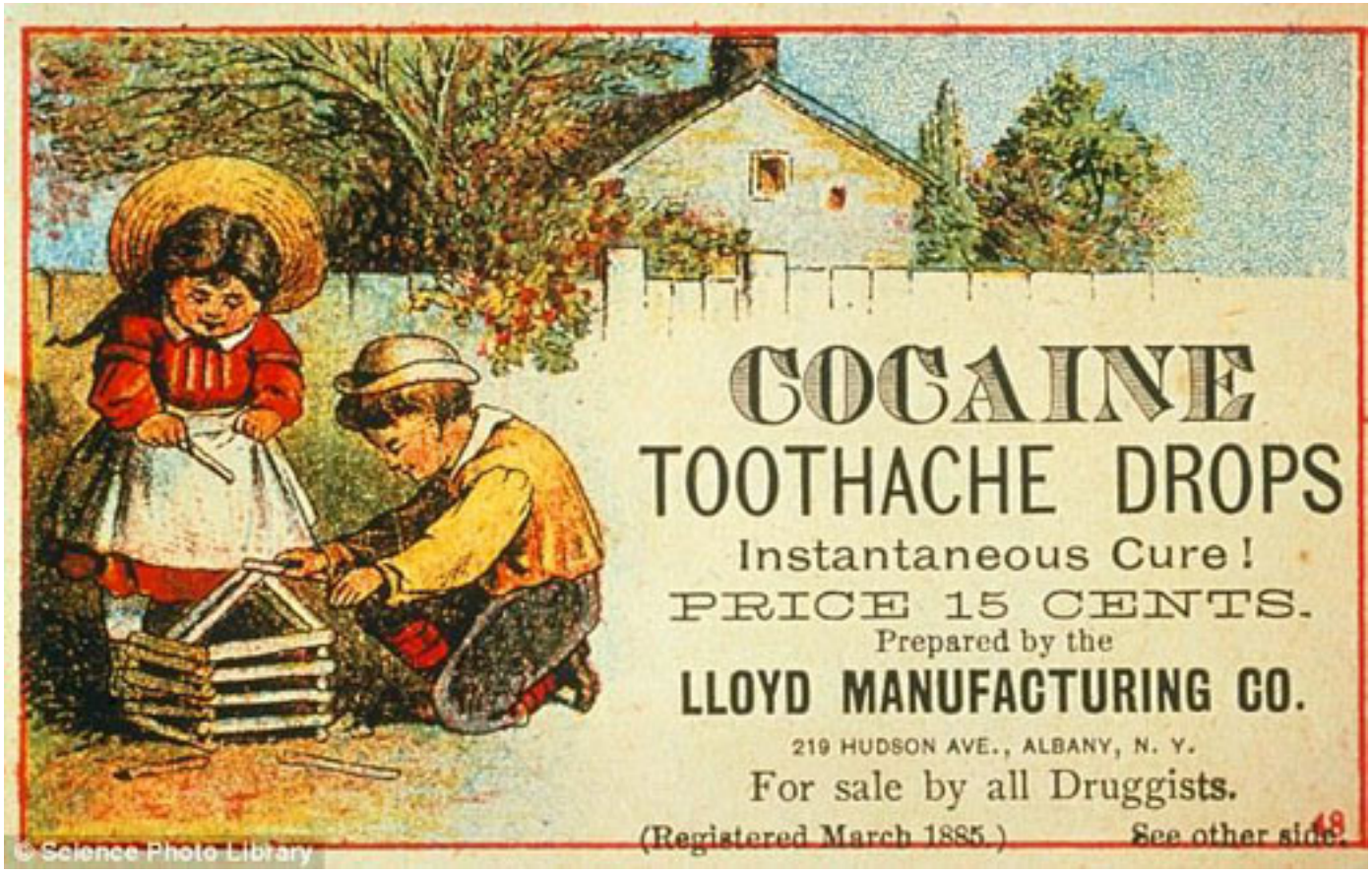
COX-1 Inhibited by Aspirin

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TOOTHACHE DROPS
Instantaneous Cure!
PRICE 15 CENTS.
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For sale by all Druggists.
(Registered March 1885.)
See other side.
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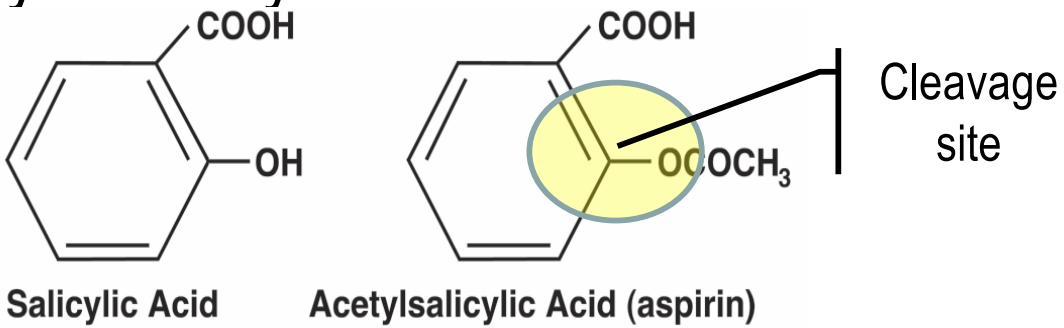
Aspirin Pharmacology

- 50% absorbed from stomach, duodenum
- Peak plasma levels at 15 minutes
- Hydrolyzed by esterase in gut, liver and RBCs
- Acetyl group hydrolyzed in 20–30 m, leave salicylic acid (measurable salicylate)
  - Platelet COX-1 acetylation occurs in the pre-systemic (portal) circulation of gut and liver
- Reduces plasma TXB_2 levels beginning within 5 m
  - Max reduction in 30 m
- Reduces urinary 11-dehydrothromboxane B2 in 2 h



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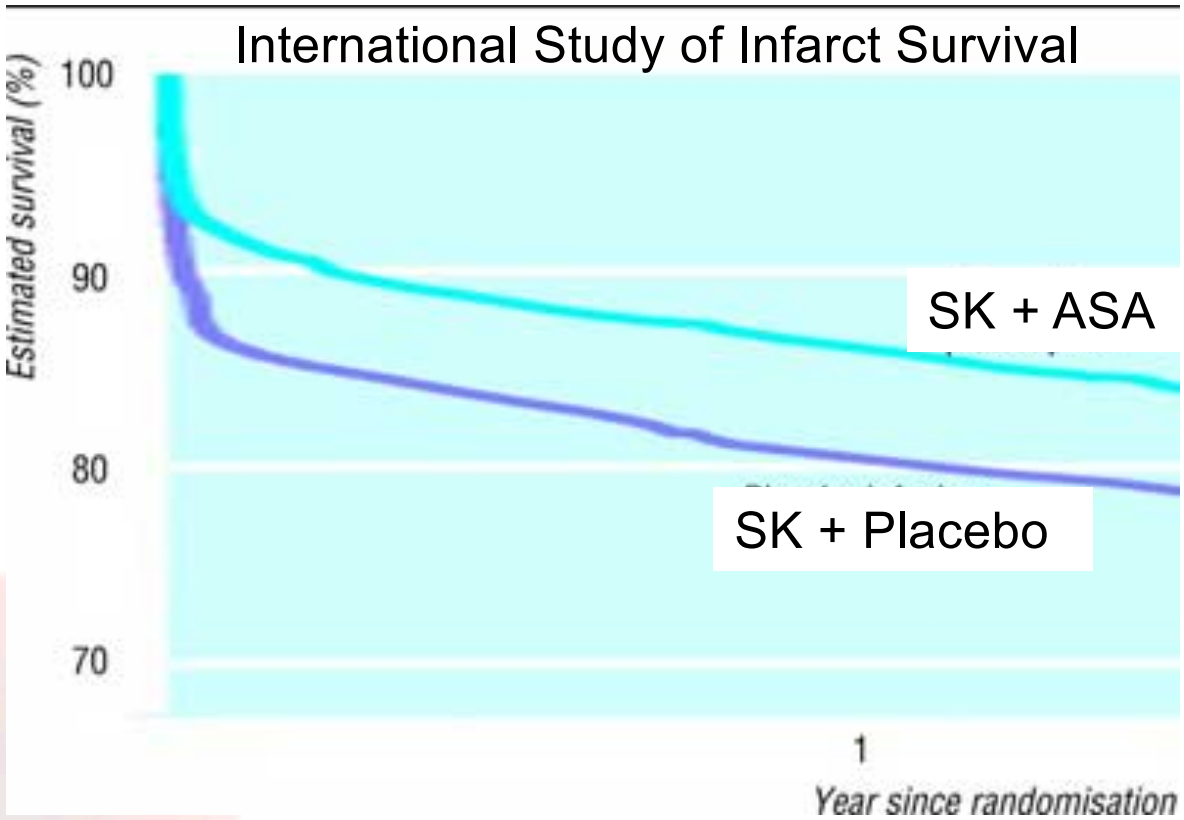
Aspirin Efficacy in Secondary Prevention: ISIS-2

1988: ISIS-2 demonstrates a 0.78 incidence of death after MI with streptokinase + ASA vs SK + placebo, FDA clears ASA to reduce risk of secondary MI or a first MI in acute angina.



ISIS-2 Collaborative Group. Randomized trial of IV streptokinase, aspirin, both or neither among 17,187 cases of suspected AMI: ISIS-2. Lancet 1988; 2: 349–60.

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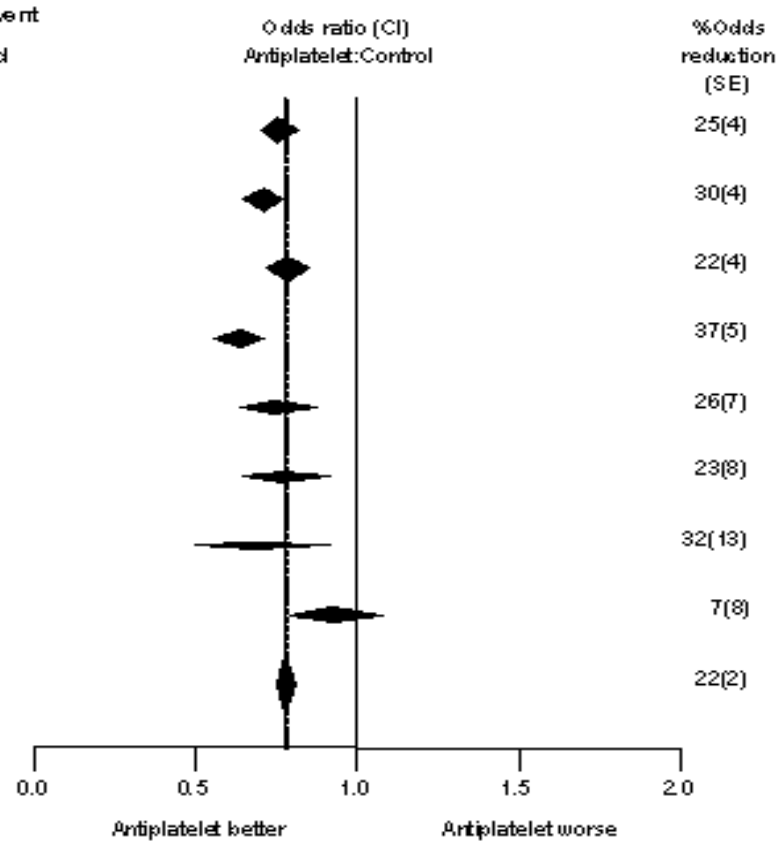
2002 Antiplatelet Trialists' Collaboration

Meta-analysis of 287 trials w/ 100,000 high-risk patients: composite 32% decrease in death, MI, ischemic stroke in vascular patients on 75–150 mg aspirin daily:

Category of patient	No of trials with data	No of patients	Percent with vascular event: Allocated antiplatelet	Percent with vascular event: Adjusted control
Previous myocardial infarction	12	20000	13.5	17.0
Acute myocardial infarction	15	19300	10.4	14.2
Previous stroke/TIA	21	12000	17.8	21.4
Coronary artery disease	55	17500	6.2	8.9
High risk of embolism	14	5200	13.5	16.8
Peripheral arterial disease	42	9700	5.8	7.1
Other vascular conditions	20	3400	4.2	6.4
Diabetes mellitus	9	5100	15.7	16.7
ALL TRIALS	195	144100	10.7	13.2



Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324: 71-86.

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Aspirin Dosage per Indication

- 75 mg (or baby aspirin—81 mg)
  - Primary and 2º MI and peripheral artery disease prevention, stroke prevention in atrial fibrillation, 2º prevention of TIA and stroke
  - Prevent pre-eclampsia, support fetal retention in primary antiphospholipid syndrome (with low MW heparin)

- 300 mg (or adult—325 mg) subsequent to:
  - MI, acute unstable angina, acute TIA, acute ischemic stroke

Navaratnam K, Alfirevic A, Alfirevic Z. Low dose aspirin and pregnancy: how important is aspirin resistance? BJOG 2016: 123: 1481–7.

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Aspirin Efficacy in Primary Prevention

- Physician's Health Study 1982–96 (♂s only)
  - 1086 healthy ♂ physicians, 40–84
  - 325 mg aspirin on alternate days versus placebo
  - 44% reduction of fatal or nonfatal first MIs
  - Ethical termination at 60 months, 1987
  - ASA cleared in 1988 to prevent TIAs and strokes in healthy ♂s >50
- Women's Health Study 1991–2000 (♀s only)
  - 39,876 healthy health care ♀s over 45
  - 100 mg aspirin on alternate days versus placebo
  - 25% reduction in fatal or non-fatal first MIs
  - But 50% reduction in smokers, hypertensives, hypercholesterolemia, greatest effect >65 YO

Physician's health study: aspirin and primary prevention of coronary heart disease. N Engl J Med 1989; 321:129-35,183-5.

Gaziano JM, Skerrett PJ, Buring JE. Aspirin in the treatment and prevention of cardiovascular disease. Haemostasis 2000; 30:1-13S.

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2018: Weight-based ASA Dosages Affect Odds Ratio of Primary CAD

Mass	ASA mg/d	Primary CAD OR	Comment
50–69 kg	75–100	0.75 (P= .007)	>100 mg ASA *raises* CAD risk!
>70 kg	75–100	0.95 (non-sig)	75–100 mg *raises* CAD risk to 1.33 (P= .0082)!
	>325	↓ (P= .017)	
Height data match weight data, findings similar in ♂ and ♀ Worldwide, 80% of men and 50% of women are >70 kg In >70 YO, ASA *raised* 3Y Ca risk by OR 1.2 (P= .02) more later			

Rothwell PM, Cook NR, Gaziano JM, et al, Effects of aspirin on risks of vascular events and cancer according to bodyweight and dose: analysis of individual patient data from randomised trials. Lancet 2018;392:387–99.

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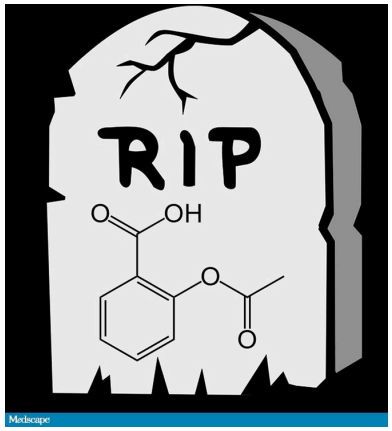
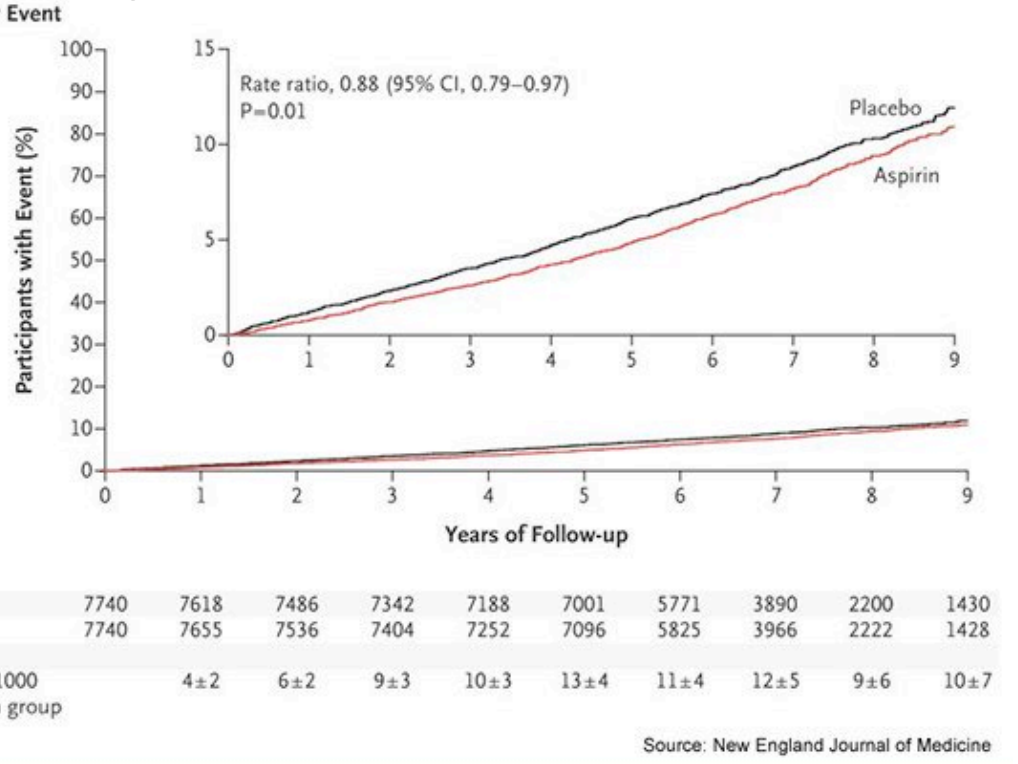
9/22/18: *ASA Offers No Protection*

- Measured vascular events: CAD, PAD, ischemic stroke
- Questionable protection for those with thrombotic risk factors
- No protection for those without cardiac indications
- Bleeding risk outweighs protection





Gaziano JM, Brotons C, Coppolecchia R, et al. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. Lancet 2018;392:1036–46.

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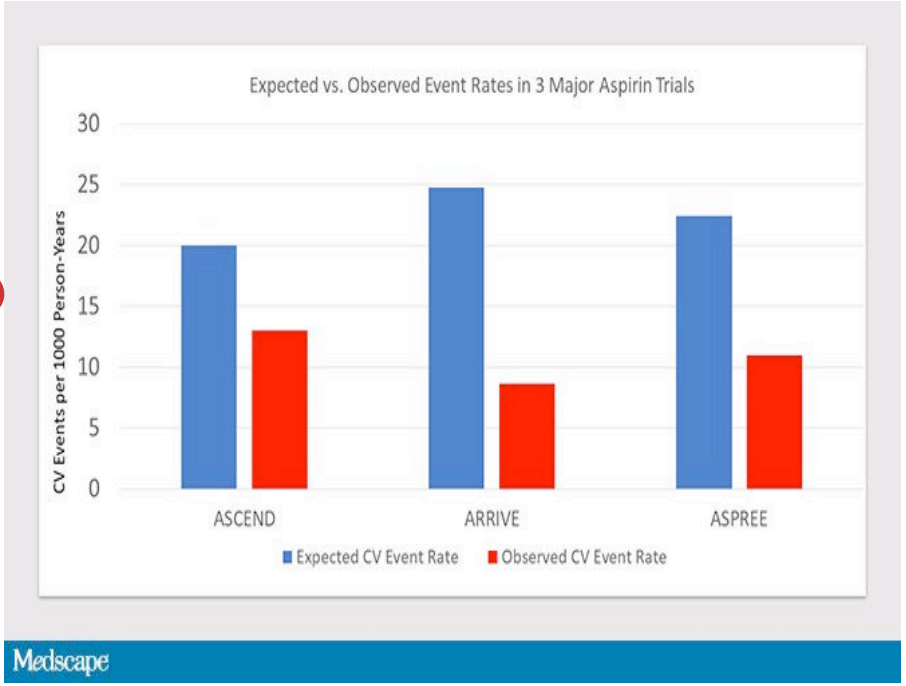
ASCEND, ARRIVE, and ASPREE Trials

- NEJM 9/16/18: 20,000 >70 in US & Australia
- Primary outcome composite of death, dementia, and disability—no effect
- ASA actually worse in…
  - All-cause mortality
  - Cancer-related death

Why the change?

- Modern CAD outcomes negate ASA value?
- CAD redefined?



McNeil JJ, Woods RL, Nelson MR, et al. Effect of aspirin on disability-free survival in the healthy elderly. N Engl J Med 2018;379:1499–1508.

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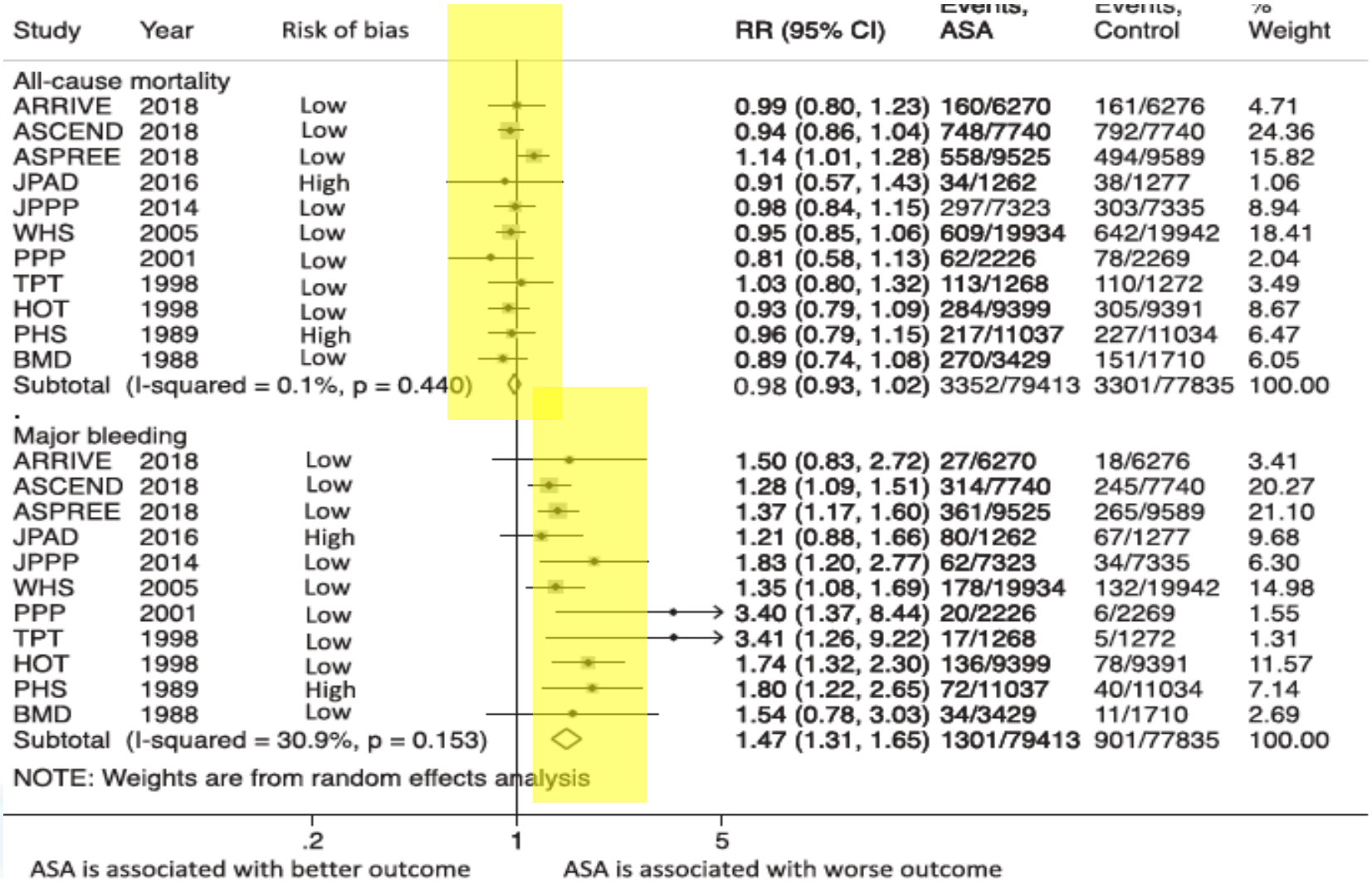
The Remarkable Story of a Wonder Drug, Which Now Comes to an End in the Primary Prevention Setting: Say Bye-bye to Aspirin!



Mahmoud AN, et al. Efficacy and safety of aspirin for primary prevention of cardiovascular events: a meta-analysis and trial sequential analysis of randomized controlled trials European Heart Journal 2019;40, 607–17.

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What is Lacking in Aspirin Studies?

Labs!

Why?

- No guideline advocating for aspirin lab assay
- Lab assays are surrogates for outcomes
- VerifyNow and PFA-100 results not reproducible
- Lab assay platform results differ among patients
- But what if you dosed on lab results?

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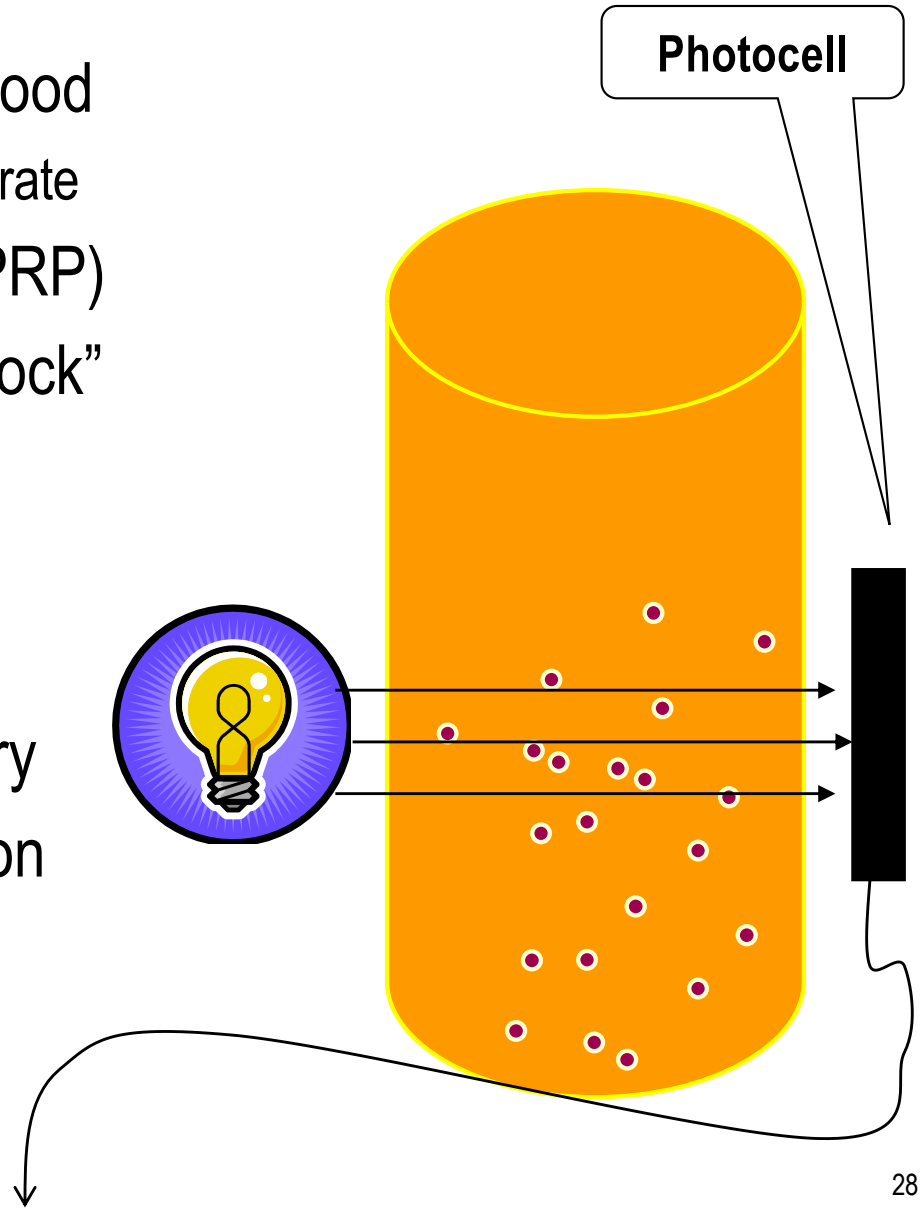
Aspirin Resistance Monitoring

- Aspirin resistance
  - Laboratory phenomenon: suboptimal platelet function suppression
- Aspirin failure
  - Secondary stroke, MI, peripheral artery disease while on ASA Rx
  - Adverse thrombotic events such as fetal loss in antiphospholipid syndrome despite aspirin therapy

How to monitor aspirin?

Light Transmittance Aggregometry (LTA) Specimen Preparation

- Collect 9–12 mL whole blood
  - 3–4 2.7 mL tubes + 0.3 citrate
- Centrifuge at 50×g 30” (PRP)
- Wait 30 m for “platelet shock”
- Dispense to cuvette
- Test within 4 hours
- Pipette agonist, record absorbance by photometry
- Attempt to record secretion by analyzing “lag phase”



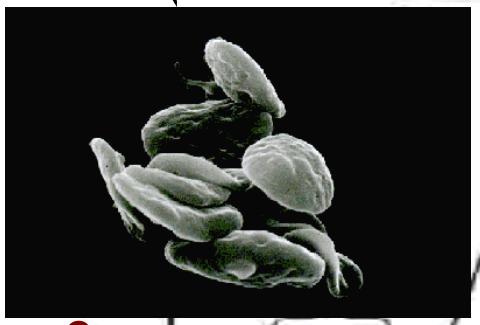
Light Transmittance Aggregometry

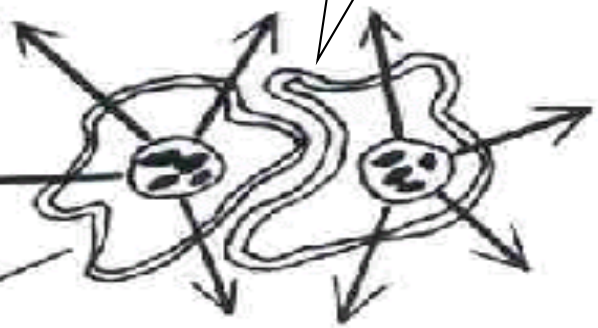
Resting Platelets

Shape Change

Primary Aggregation

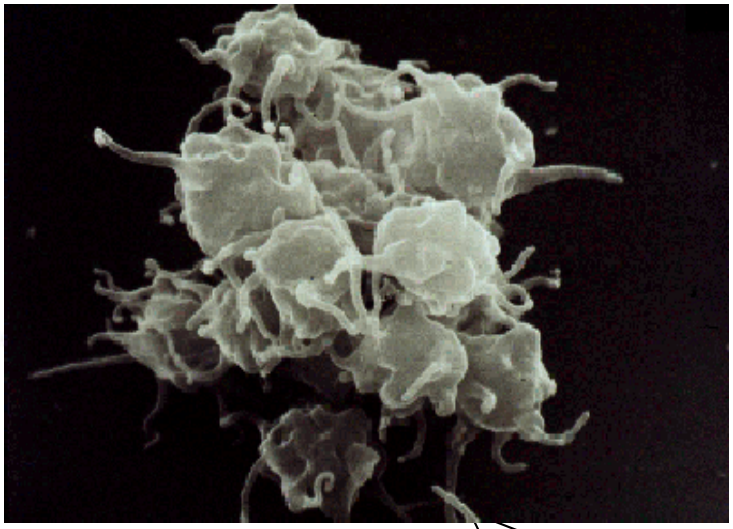
Secretion





0% Transmittance

Agonist



100% Transmittance

0

1 MINUTE

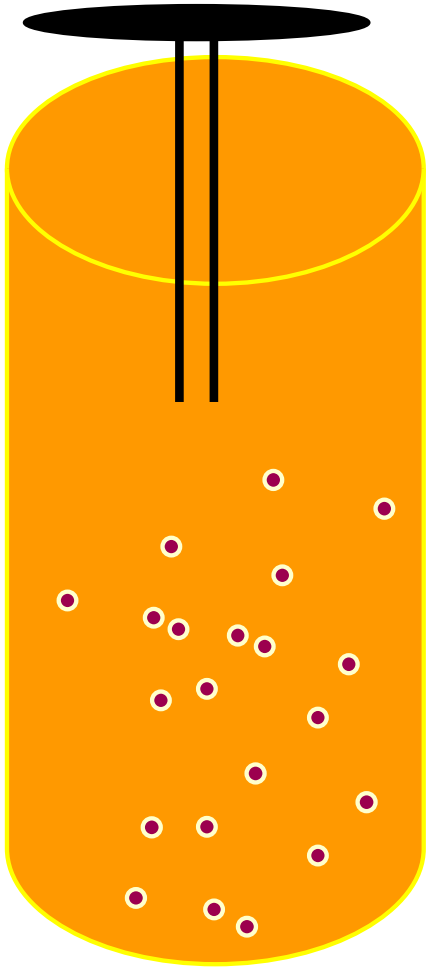
5 minutes

Secondary Aggregation

Courtesy of Kathy Jacobs, Chronolog, Inc

Impedance-based Whole Blood Aggregometry (WBA)

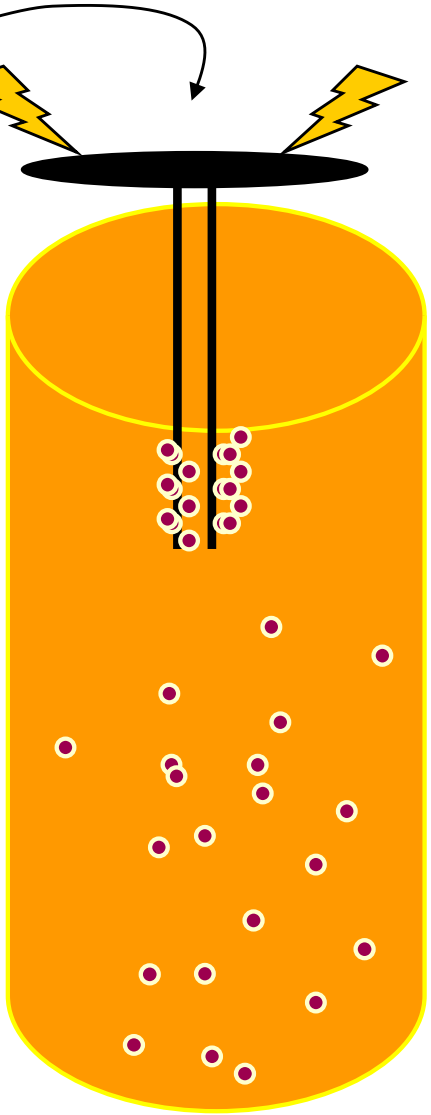
- Collect 9 mL blood, do not centrifuge
  - 3 tubes each 2.7 mL + 0.3 citrate
- Aliquot, dilute 1:1 with saline
- Pipette agonist, timer starts
- Electrodes lowered into suspension

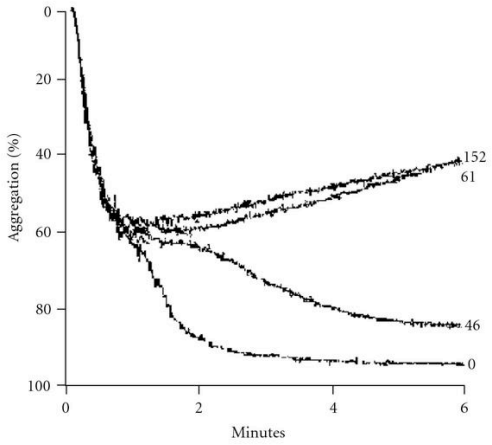


Courtesy of Kathy Jacobs, Chronolog, Inc

WBA: Impedance

- Aggregating platelets form layer on electrodes
- Platelet layer impedes current
  - Resistance in ohms (Ω)
  - 0 Ω = no aggregation
  - Aggregation proportional to Ω
  - Same pattern as LTA





Courtesy of Kathy Jacobs, Chronolog, Inc

Aspirin Efficacy Agonists

- 0.5 mM arachidonic acid (AA)
  - Directly activates eicosanoid synthesis pathway to produce TXA_2
  - TXA_2 activates platelet by binding internal receptors TPα or TPβ
  - Response reduced by aspirin
- 1–5 μg/mL collagen
  - Binds receptors GP Ia/IIa (integrin α2β1), GP IV, GP VI
  - Response reduced by aspirin
  - May bypass aspirin effect, aggregation via alternate pathways
- 5–10 μM ADP
  - ADP binds P_2Y_{12} receptor
  - Response reduced by thienopyridines like Plavix, Brilinta
  - May bypass aspirin effect, aggregation via alternate pathways

Secretion Response Using The "Firefly" Reaction

ATP → PPi

Luciferin → Luciferyl:AMP

Chromo-lume® firefly luciferase reagent

O_2

Luminescence

Proportional to ATP release in ηM

Oxyluciferin + AMP

Courtesy of Kathy Jacobs, Chronolog, Inc

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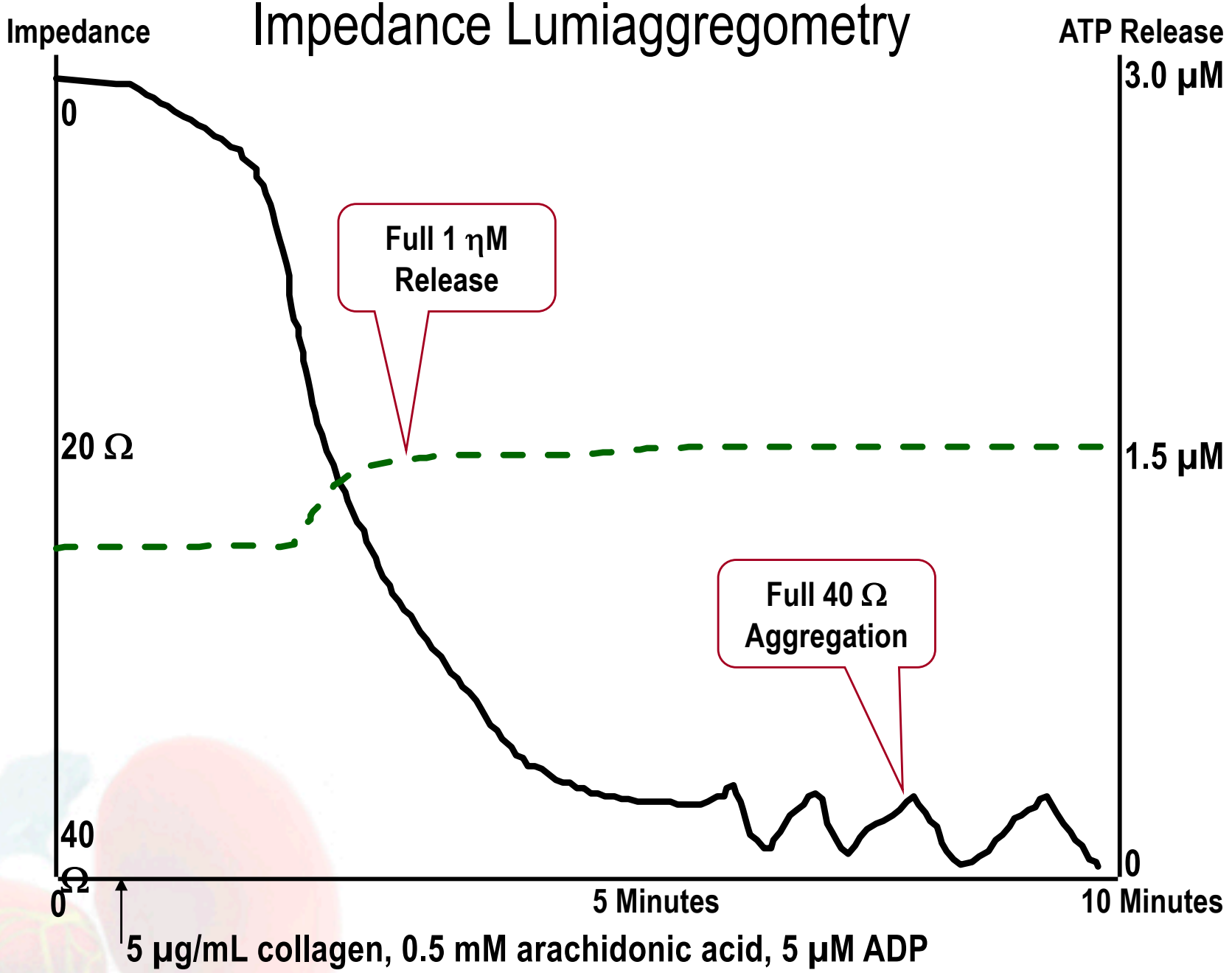
Normal Whole Blood Impedance Lumiaggregometry



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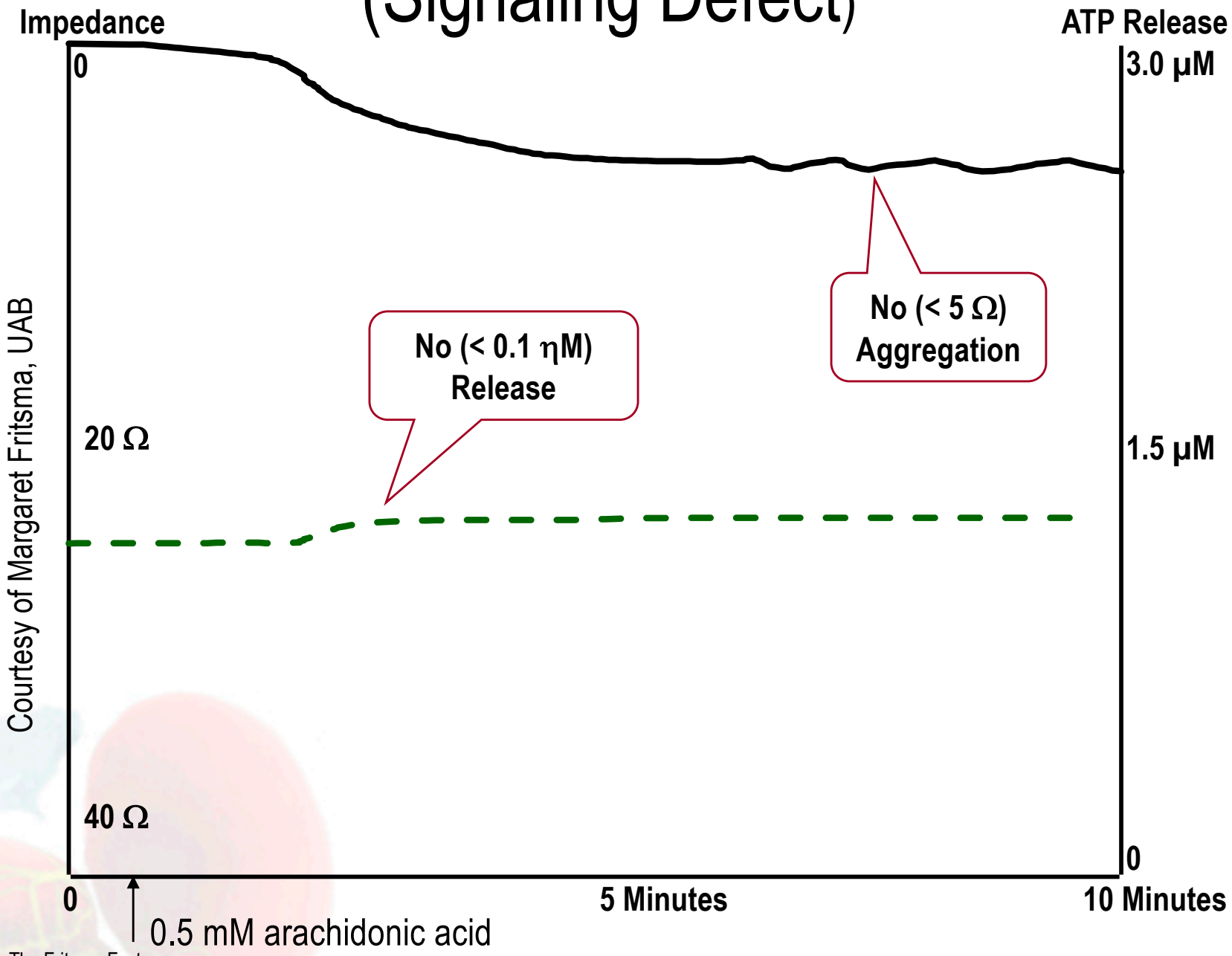
Aspirin or "Aspirin-like Disorder" (Signaling Defect)



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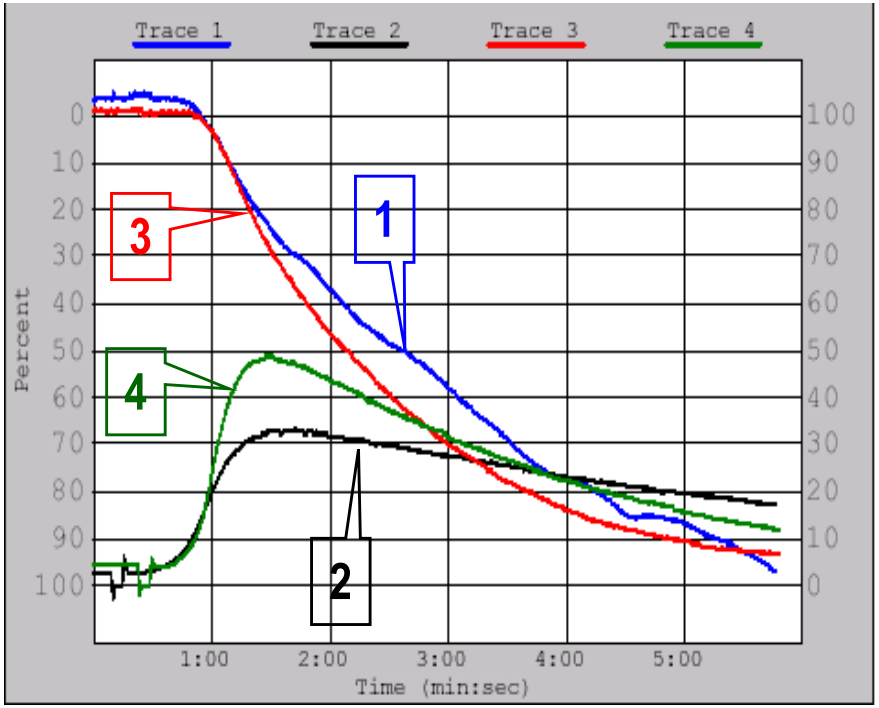
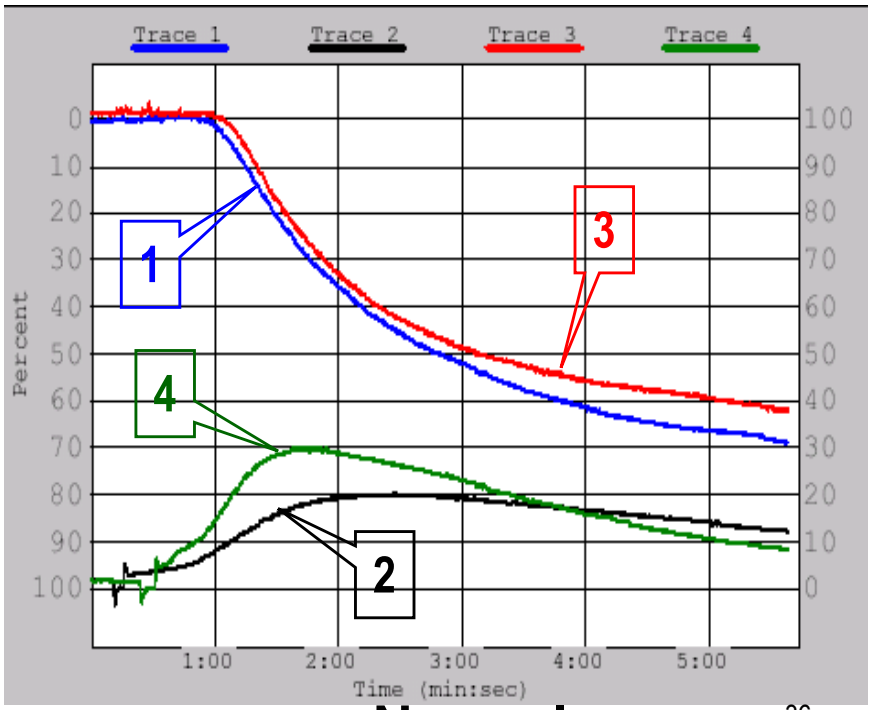
Agonist	Collagen 5 μg/mL	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	0.83ηm	0.81ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	38Ω	37Ω



Agonist	Arachidonic Acid 0.5 mM	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	0.45ηm	0.45ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	27Ω	25Ω



Courtesy of Patti Tichenor, UAB Coagulation; using AGGRO/LINK®

Normal

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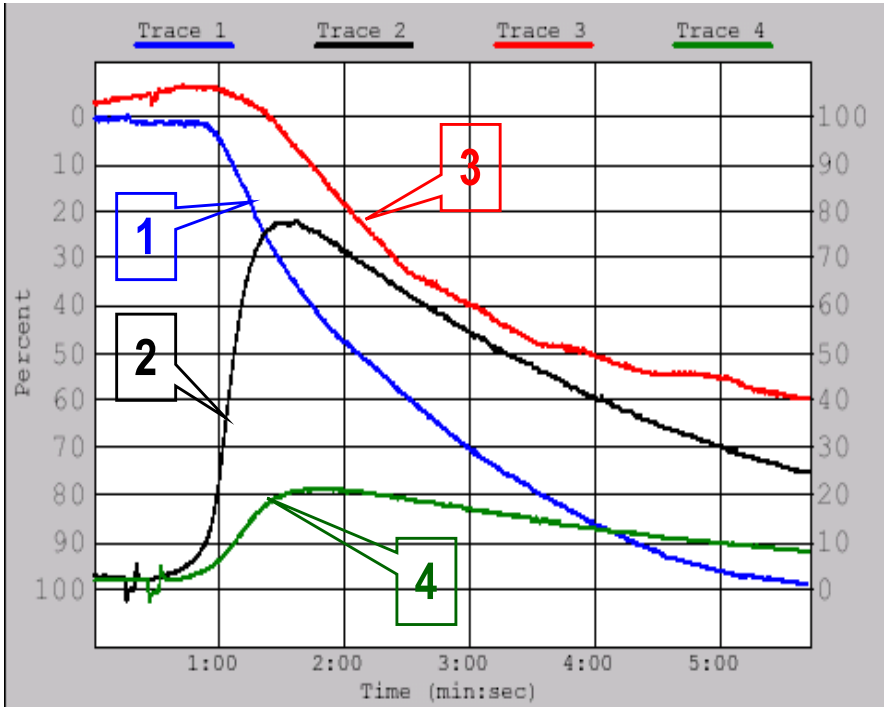
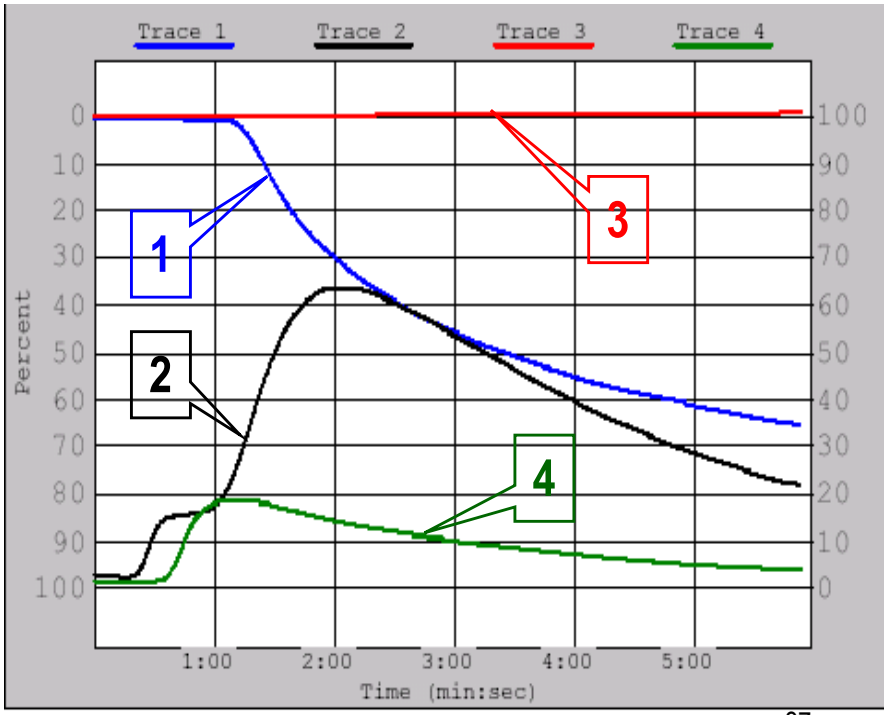
Agonist	Collagen 5 μg/mL	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	1.69ηm	0.57ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	39Ω	26Ω



Agonist	Arachidonic Acid 0.5 mM	
Tracing	2-Control	4-Patient
Luminescence (Amplitude)	1.42ηm	0.57ηm
Tracing	1 Control	3-Patient
Aggregation (Impedance)	26Ω	0Ω



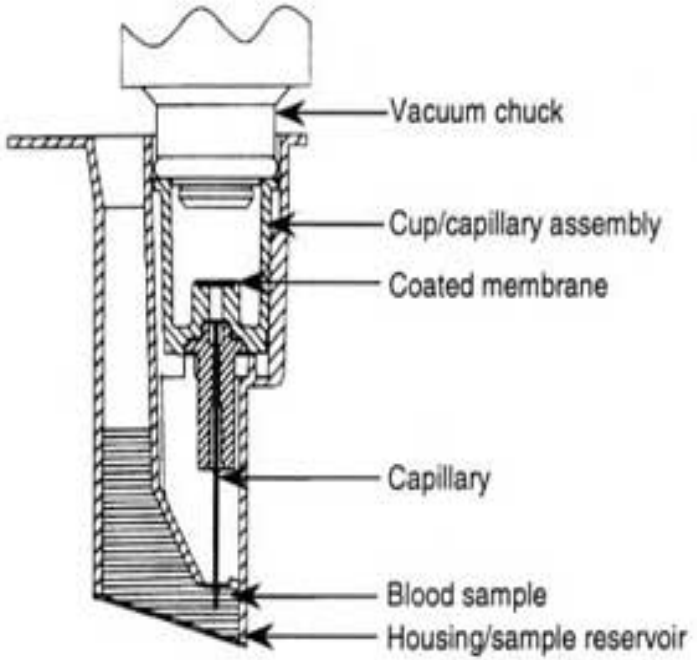
Courtesy of Laura Taylor, UAB

Coagulation; using AGGRO/LINK®

Aspirin

Siemens (Dade-Behring) PFA-100

- Transfer 800 μL citrated whole blood to each of two cartridges, load and run to closure time (CT)
  - Coated membrane: agonists
- Collagen and epinephrine (Col/Epi, CEPI)
  - "Weak:" normal CT 98–185 s
  - Initial cartridge
- Collagen and ADP (Col/ADP, CADP)
  - 50 μM ADP
  - "Strong:" normal CT 77–133 s
  - Confirmatory cartridge



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PFA-100 Aspirin Response Assay

PFA-100 does not use arachidonic acid

Col/Epi Cartridge

CT >185s

Col/ADP Cartridge

CT >125s

CT <185s

If ASA present and CEPI CT is <185s, aspirin resistance

CT <125s

Normal or aspirin resistance

- Severe VWD
- Severe PLT dysfunction

- Aspirin, NSAIDs
- Reduced HCT or PLT count
- Mild PLT dysfunction
- Mild VWD

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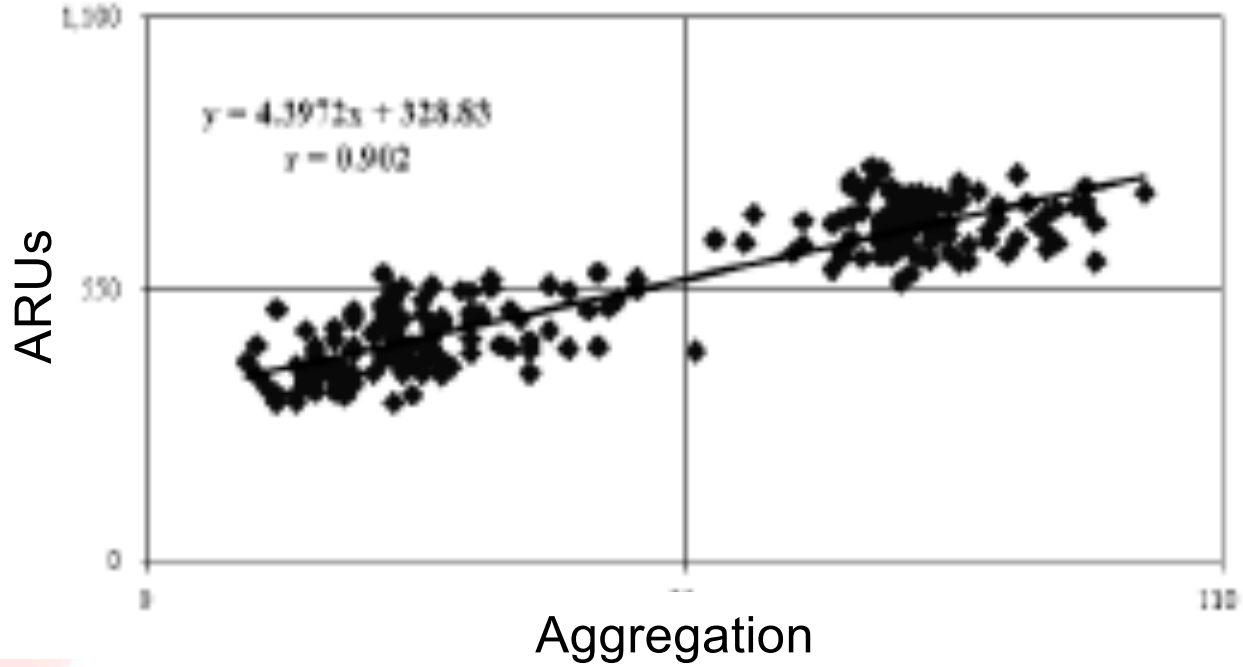
Werfen VerifyNow®
Aspirin Resistance Units (ARU)

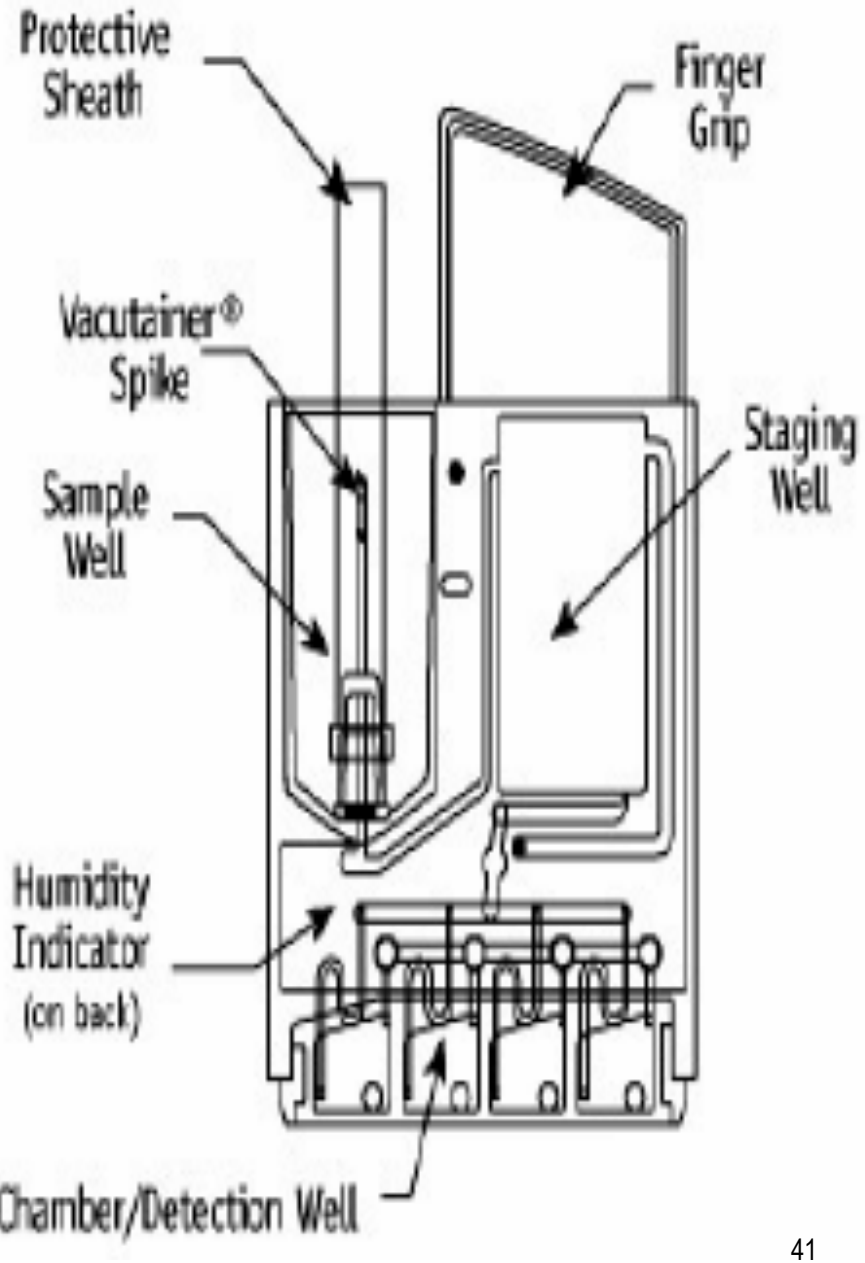
Whole blood light transmittance rises as platelets aggregate to arachidonic acid. Aggregation suppressed by aspirin:

- ARUs <550: function inhibited = aspirin sensitive
- ARUs >550: function normal = aspirin resistance

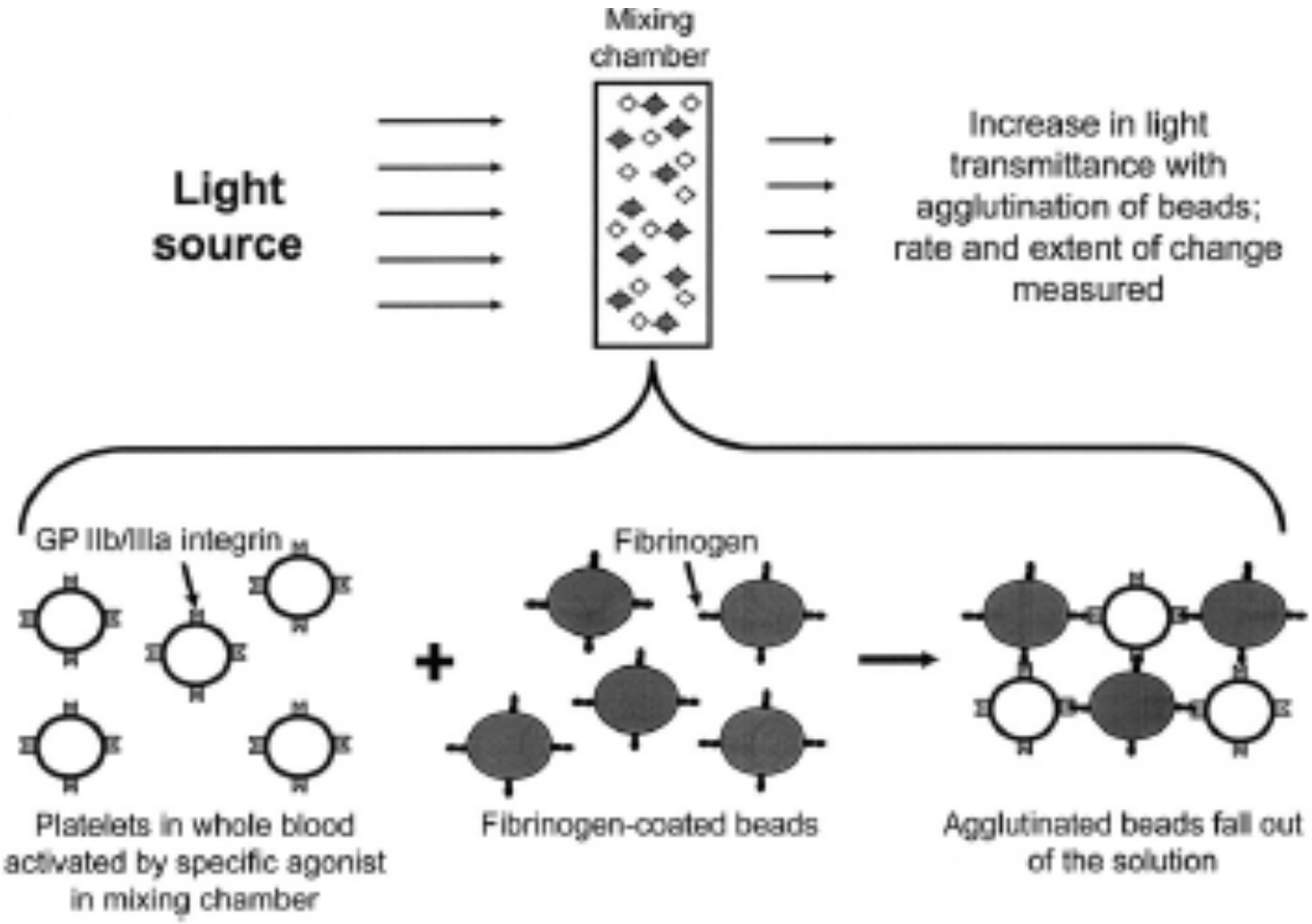


Werfen VerifyNow Reaction Chamber

ARUs measure aspirin resistance



VerifyNow Principle



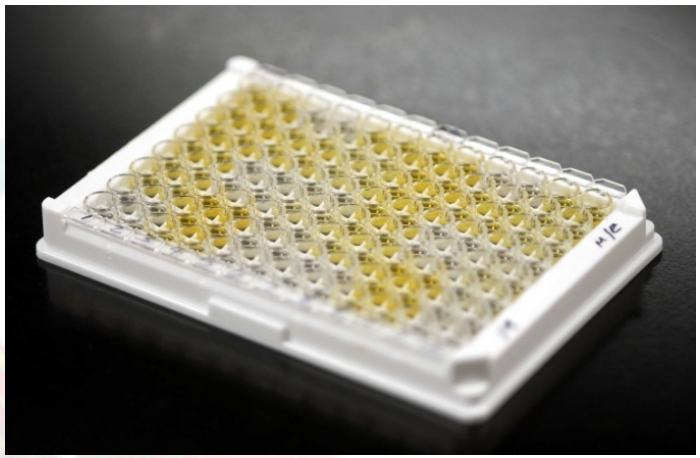
Werfen VerifyNow and PFA-100 Limitations

- Cartridges ~$5.00 each
- Whole blood specimen volume 800 µL/assay
- Precision: CVs >10%, often requires duplication
- Must test whole blood within four hours
- Variable effects of…
  - von Willebrand factor, factor VIII, fibrinogen
  - Platelet count and hematocrit

McGlasson DL, Fritsma GA, Shah AD. Effects of elevated fibrinogen, factor VIII, von Willebrand antigen, and immunologic von Willebrand factor on the INNOVANCE® PFA P2Y cartridge. Poster, ISTH 2009

AspirinWorks®
a division of Creative Clinical Concepts, Inc.

- Urinary 11-dehydrothromboxane B_2 (11-DHTB$_2$)
  - Liver-produce metabolite of plasma thromboxane B_2
- Platelet is the primary source for 11-DHTB$_2$
  - Also renal endothelial cells, monocytes
- Random urine specimen: store and ship
  - Normalized to urine creatinine: Pg 11-DHTB$_2$ /mg creatinine
- Liver and renal disease limitations




SULPH DEP
ESS: ABSINTH S:
ESS: CENEMIN
AQ PHAGDAN
AQV:

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2002 HOPE Study: Aspirin Resistance

- Nested (quartile) retrospective case-control sample
  - 488 aspirin-treated CAD patients: end point was 5-yr secondary MI, stroke, or CV death
  - 488 age- and sex-matched controls taking aspirin who did not have an MI, stroke, or CV death
- In aspirin-treated CAD patients, 11-DHTB$_2$ predicts risk of MI or CV death: fourth quartile 11-DHTB$_2$ = OR 3.5 for CV death

Pg 11-DHTB$_2$/ mg creatinine	Quartile	Odds Ratio		
		MI	CV Death	Stroke
<134	1	1.0	1.0	1.0
134–193	2	1.3	2.0	2.5
194–298	3	1.5	2.5	0.6
>298	4	2.0	3.5	0.6

Eikelboom JW, Hirsh J, Weitz JI, et al. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002; 105: 1650–55

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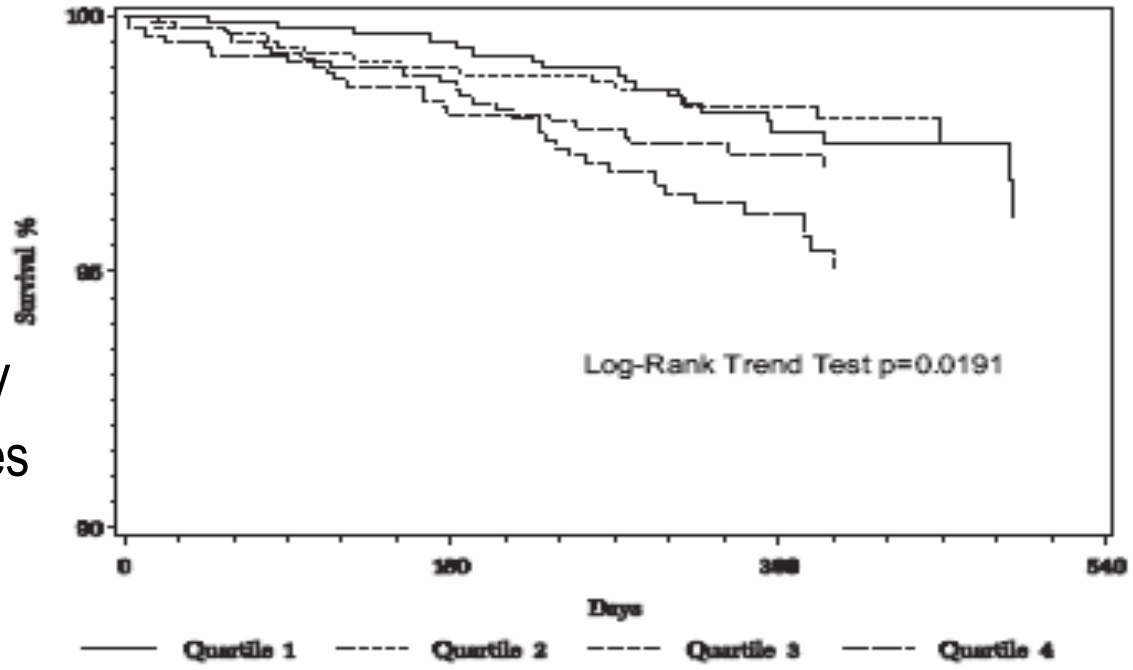
CHARISMA Trial: Dual Antiplatelet Therapy

- Randomized double-blind prospective trial of 3261 clopidogrel Vs. placebo in patients on aspirin at high risk of athero-thrombosis
  - Tested 1 month after starting clopidogrel, assigned quartiles
  - 144 with one-year stroke, MI, or CV death
  - 3117 with no adverse event
- Fourth quartile 11-DHT_2 composite OR=1.66

Kaplan–Meier curves for composite of stroke, MI, or CV death by quartiles



Eikelboom JW, Hankey GJ, Thom J, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid. Determinants and effect on cardiovascular risk. Circulation 2008; 118: 1705–12

CHARISMA Trial Conclusions

- Fourth quartile normal or elevated 11-$DHTB_2$
  - Signifies aspirin resistance
  - Age, ♀, PAD Hx, smoking, oral hypoglycemic Rx, ACE-inhibitor Rx
- Reduced 11-$DHTB_2$
  - Aspirin Rx >150 mg/d, NSAIDs, hypercholesterolemia, statin Rx
- Randomization to clopidogrel or placebo did not reduce risk ratio for CV events in patients in the fourth 11-$DHTB_2$ quartile
- 11-$DHTB_2$ level is potentially modifiable

Aspirin Resistance Prevalence

Overall		27.1%
By definition	PFA-100	29.0%
	Ultegra VerifyNow	26.2%
	LTA	21.3%
By population	CAD	22.9%
	Stroke	32.1%
By dose	< 100 mg/d	35.6%
	101–299 mg/d	28.2%
	> 300 mg/d	18.6%

Hovens MMC, Snoep JD, Eikenboom CJ. Prevalence of persistent platelet reactivity despite use of aspirin: a systematic review. Am Heart J 2007;153:175–81.

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Aspirin Resistance and Adverse Events Review

Type	Percutaneous Intervention (Cath)		Stable CAD
N	151	106	315
% AR	19.2%		5.2%
Method	VerifyNow	Light Transmittance Aggregometry	
Results	Elevated creatine kinase and troponin I in AR	4th quartile ADP response associated with OR for CV events = 22.4	OR in AR Composite: 3.12 CV death: 2.98 MI: 1.91 CVA: 5.44
Ref	Chen WH, JACC 2004;43:1122	Cuisset T, J Thromb Haemost 2006;4:542	Gum PA, JACC 2003;41:961

AR= ASA resistance; CAD= coronary artery disease; OR= odds ratio; CV= cardiovascular; MI= myocardial infarction; CVA= cerebrovascular event

Siemens PFA-100 and Aspirin Resistance

Gum PA, JACC 2003;41:961	9.5% aspirin resistance by CEPI closure time, poor correlation with LTA
Hézard N, Thromb Res 2002;108:43	Poor aspirin resistance correlation among LTA, CEPI closure time, and flow cytometry (P-selectin)
Sane DC. Thromb Haemost 2002;88:711	No CEPI closure time difference between aspirin resistance and aspirin sensitive
Ten Berg JM, Thromb Res 2002;105:385	CEPI closure time does not distinguish low dose from high dose aspirin
Grundmann K, J Neurol 2003;250:63	53 patients on aspirin for stroke prevention: CEPI closure time significantly shorter in 12/35 patients with recurrent stroke ($p < 0.01$)
CEPI = collagen-epinephrine cartridge LTA = light transmittance aggregometry	

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Variation in Lab Detection of Aspirin Resistance

Assay	Aspirin Resistance %
Werfen VerifyNow Aspirin	17
Siemens PFA-100 CEPI	22
LTA	5
All tests abnormal per subject	2
Harrison P, Segal H, Blasbery K. Screening for aspirin responsiveness after transient ischemic attack and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. Stroke 2005 36:1001–5.	

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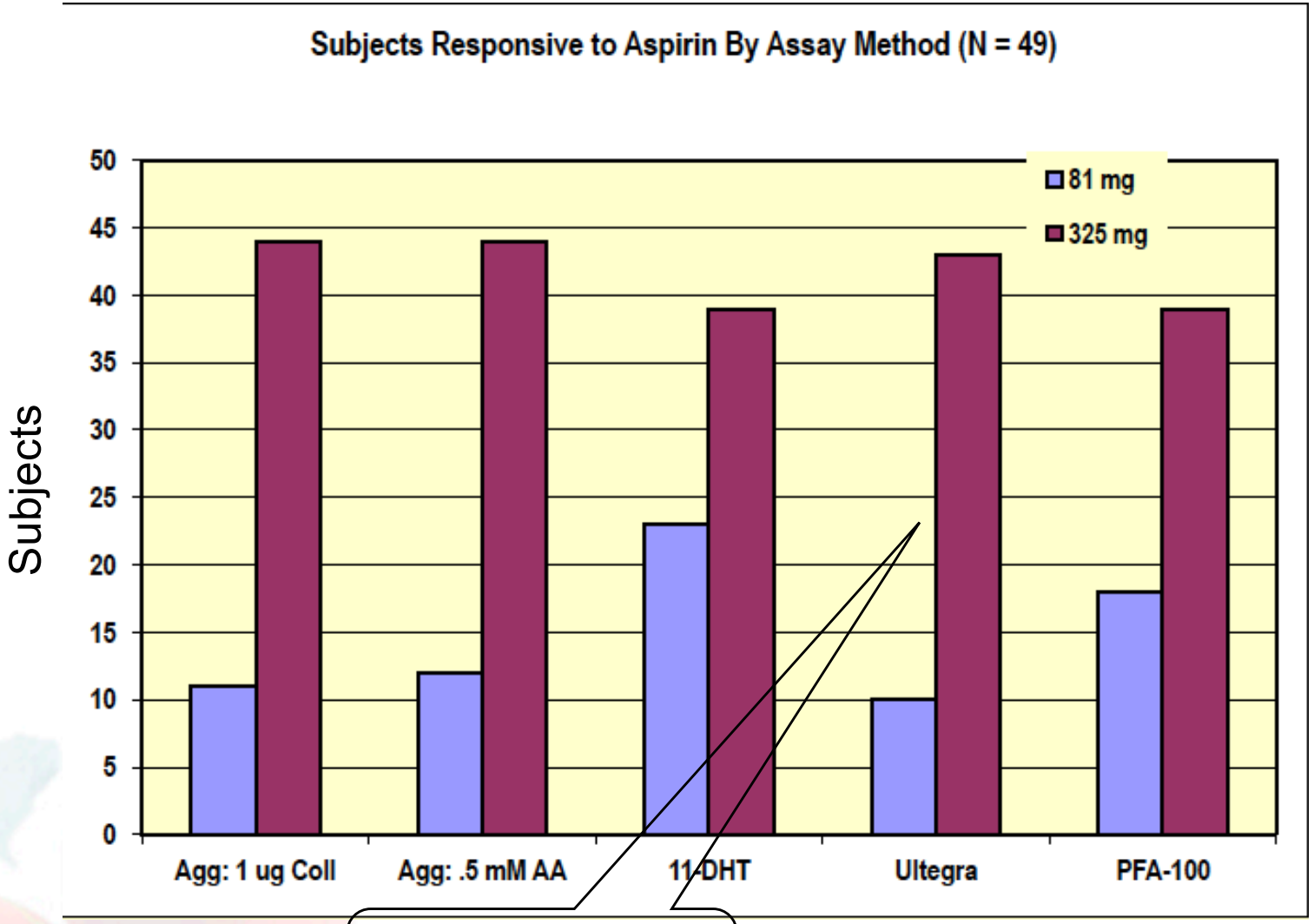
24-h Response to 81 & 325 mg ASA



Aspirin Seven Days: Test Efficacy
Comparison to Whole Blood Aggregometry

	Positive Predictive Value		Negative Predictive Value	
Dosage	81 mg	325 mg	81 mg	325 mg
11 DehydroTXB$_2$	74.3%	82.1%	40.2%	0.0%
PFA-100 CEPI	81.3%	81.6%	53.8%	42.9%
VerifyNow Aspirin	72.7%	51.9%	100%	33.3%

"Laboratory measures of PLT activity are suppressed by ASA Rx, and are affected by the dosage and duration of therapy. Determinations of aspirin response should be made after at least 7 days of treatment. Laboratory test platform results do not closely reflect each other, thus application of laboratory platforms should be made consistently."

McGlasson DL, Fritsma GA. Comparison of four laboratory methods to assess aspirin sensitivity. Blood Coagul Fibrinolysis 2008;9:20–3

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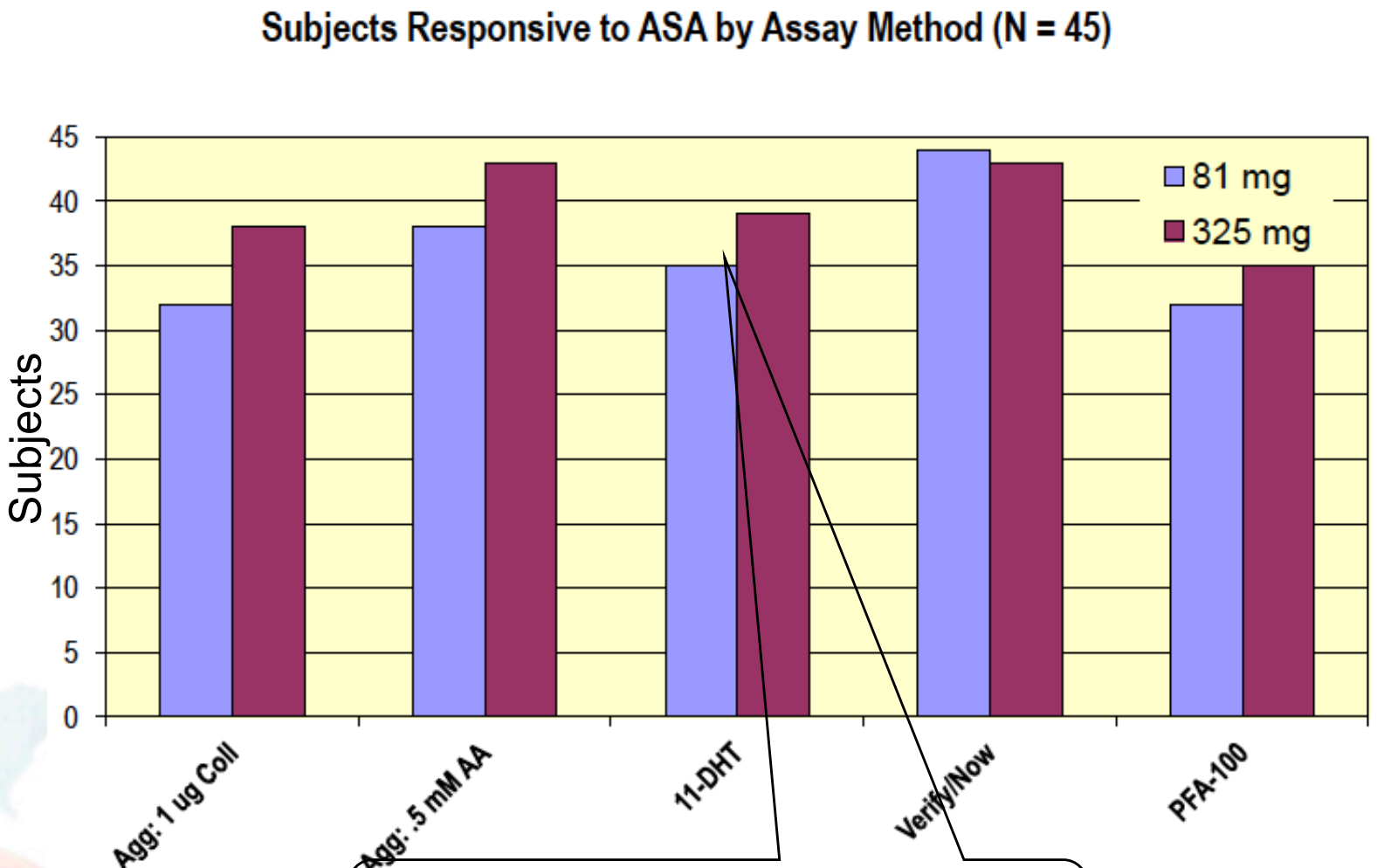
7-d Response to 81 & 325 mg



Little dosage difference but patient response is greater

Aspirin Resistance Study Limitations

- Inter-assay variation
- Biological variation over time
- Failure to adjust for race, age and sex
- Failure to confirm compliance
  - Serum salicylate?
  - Non-compliance and early withdrawal may account for most AR
- Failure to separate confounding conditions
  - Hypertension, diabetes, peripheral vascular disease, smoking, and inflammation may contribute to aspirin resistance, while independently raising vascular risk

Proposed Mechanisms of Aspirin Resistance

- Increased platelet turnover: >10%/d
- Activation of alternate platelet pathways not blocked by aspirin
  - Diacylglycerol pathway activated through G-protein
  - Adhesion molecules: collagen (GPIa/IIa) & VWF receptors (GPIb/IX/V)
  - Activation by shear stress in atherosclerosis
- Aspirin-mediated reduction of PLT-inhibiting prostacyclins from vascular endothelial cells
- Elevated VWF, fibrinogen activity level
- Polypharmacy (> 4 drugs)

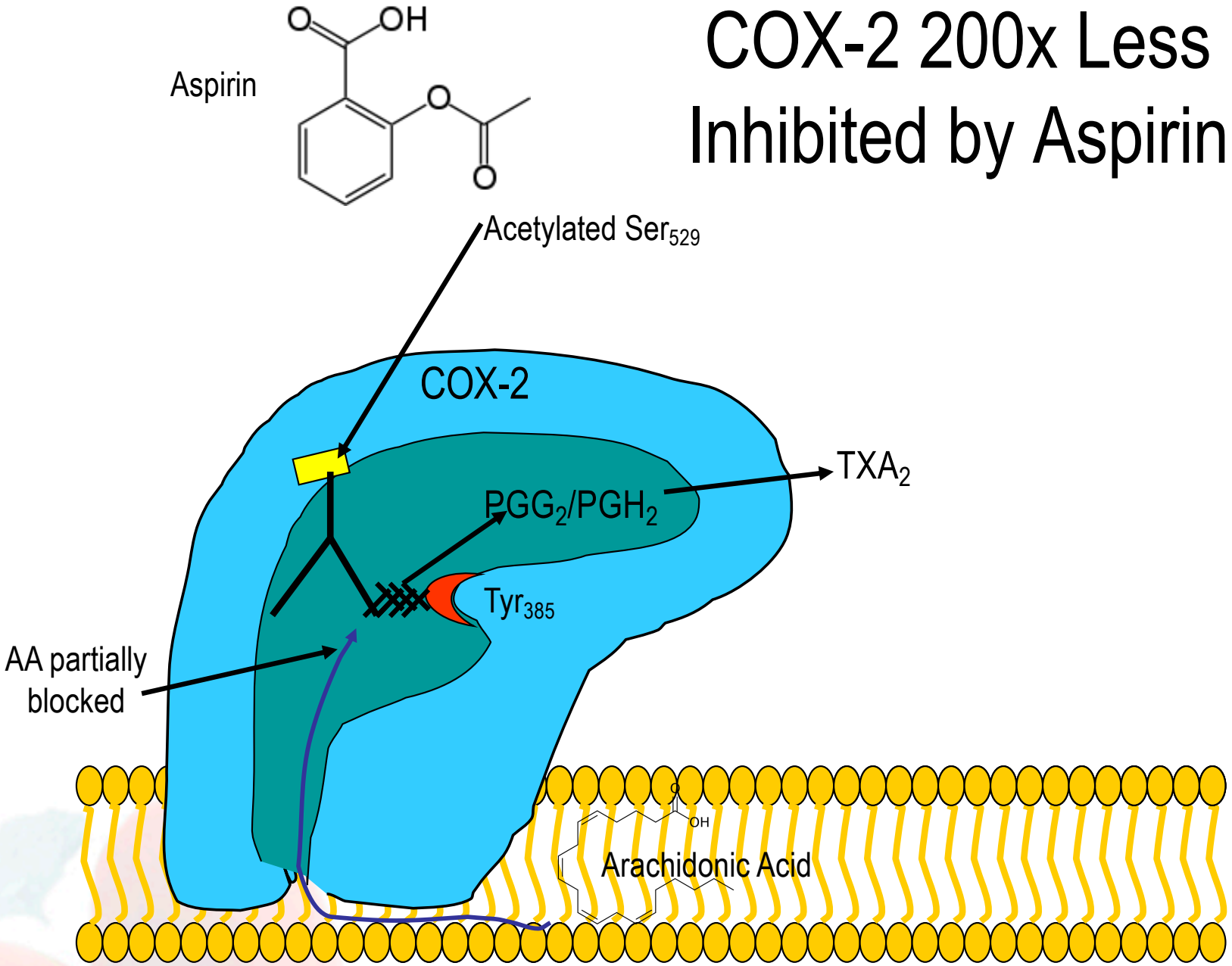
Goodman T, Sharma P, Ferro A. The genetics of aspirin resistance. Int J Clin Pract 2007:61:826–34
Kilanowska J, Favaloro EJ, Lippi G. Aspirin “responsiveness,” “nonresponsiveness” or “resistance”: a putative role for von Willebrand factor? Blood Coagul Fibrinolysis 2008;19:823–4

More Proposed Mechanisms of Aspirin Resistance

- NSAIDs compete for Ser_{529} site
  - Naprosyn, ibuprofen reversible bonds block ASA
- COX-2 Induction most likely
  - Non-constitutive, COX-2 response to cytokines and inflammation
  - Smoking, diabetes, heart failure and hyperlipidemia
  - COX-2 in megakaryocytes, monocytes, macrophages, vascular endothelial cells and newly released platelets
  - After bypass surgery, 16-fold increase of COX-2 causing transient aspirin resistance
  - Acetylation of COX-2 ser_{529} incompletely hinders arachidonic acid's access to reactive site

Weber AA, Zimmermann KC, Meyer-Kirchrath J, Schror K. Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance (letter). Lancet 1999; 353: 900.

COX-2 200x Less Inhibited by Aspirin



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Dual Antiplatelet Therapy

- Aspirin, 81 or 325 mg + clopidogrel, 75 mg; or prasugrel, ticagrelor critical in stents, reduce secondary MI 20%
- Clopidogrel resistance 15–63% detected by molecular or phenotypic tests, predicts risk of secondary MI
- Little variability in response to prasugrel or ticagrelor
- Ticagrelor is more effective than clopidogrel in ACS with and without PCI. Similar rates of bleeding as clopidogrel
- Typical Rx duration: aspirin indefinite, clopidogrel 1–2 y
- Triple therapy: ASA & clopidogrel + Coumadin or DOAC: benefit in mechanical valves, bleeding up 43%, mortality down 57%
  - Clinical management is tricky
  - Laboratory monitoring may be confounded by polypharmacy

Bridging Dual Antiplatelet Therapy During Surgery

- Discontinue: 20% incidence of ischemia
- Continue: 35% increased bleeding
- ISTH 2019 guidelines
  - Harmonize risks relative to procedure
  - Assess surgical procedure bleeding risk: min, low, mod, high
  - Assess patient thrombosis risk: low, mod, high

Spyropoulos AC, Brohi K, Caprini J, et al. Scientific and Standardization Committee Communication: Guidance document on the periprocedural management of patients on chronic oral anticoagulant therapy: Recommendations for standardized reporting of procedural/surgical bleed risk and patient-specific thromboembolic risk. J Thromb Haemost. 2019; pre-pub. DOI: 10.1111/jth.14598

So, do we test for aspirin resistance?

W Check. Clot Knot; Unraveling Tests for Coag Disorders. CAP Today, December 2008.

Dr. Kristi Smock, ARUP: “I think it is a problem of using different definitions for aspirin resistance and measuring it with tests that have different sensitivities and specificities.” “Moreover,” she adds, “testing for this condition is not generally recommended because it is not known what the treatment changes would be. “

Dentali F, Douketis JD, Lim W, Crowther M. Combined aspirin–oral anticoagulant therapy compared with oral anticoagulant therapy alone among patients at risk for cardiovascular disease a meta-analysis of randomized trials Arch Intern Med 2007;167:117–24.

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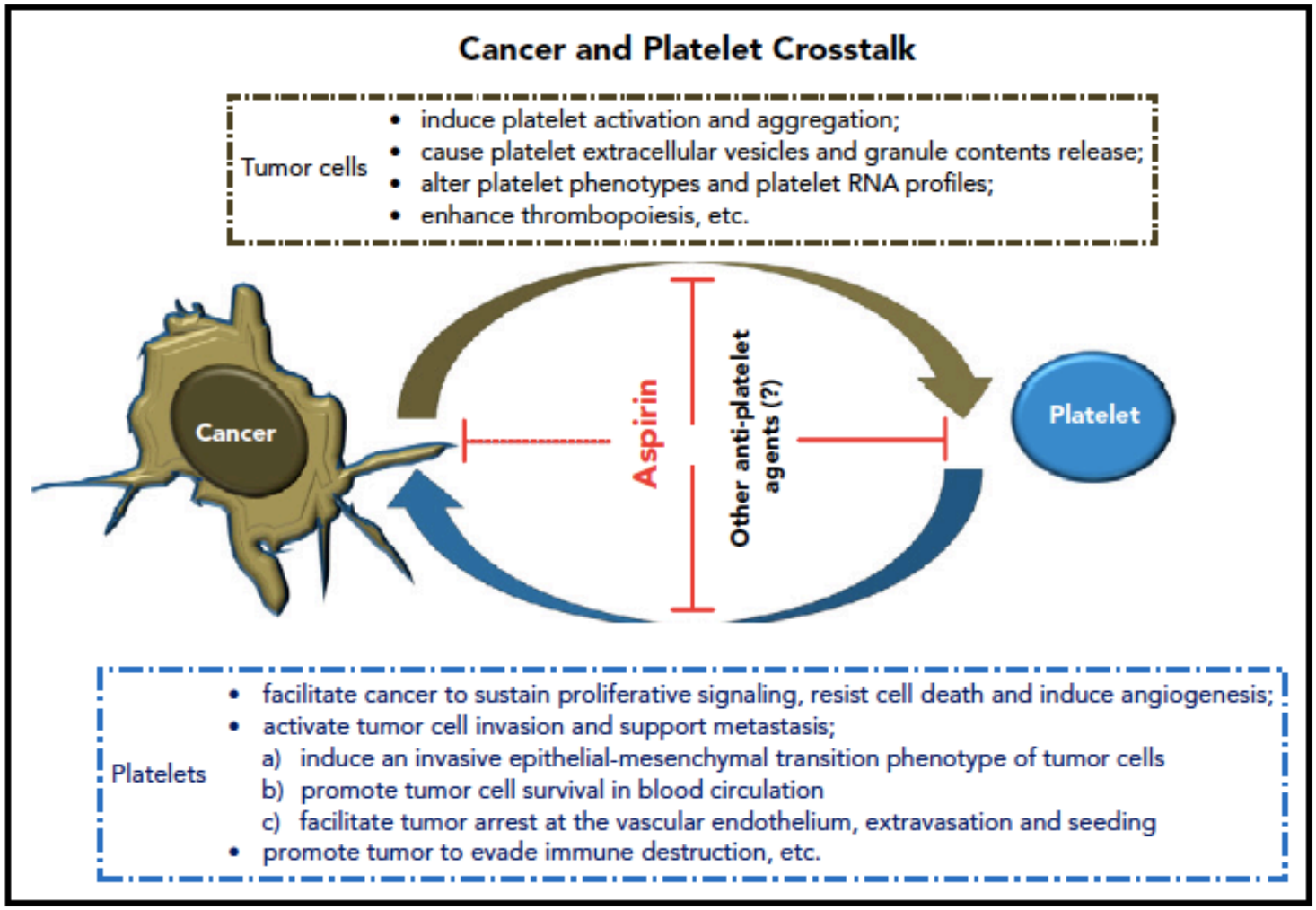
Response: Two Meta-Analyses

- Mean prevalence of laboratory ASA resistance among all methods is 27%. Resistance predicts 3.8 OR for adverse cardiovascular outcomes. No concordance among methods.
- 20 studies totaling 2930 patients with cardiovascular disease. Classified 28% as aspirin resistant. Resistance confers a 3.85 OR for any adverse cardiovascular outcome including a 5.99 OR for death and a 2.96 OR for acute coronary syndrome. No concordance among methods.

- Snoep JD, Hovens MMC, Eikenboom JCJ, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis Arch Inter. Arch Intern Med 2007;167:1593-9
- Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR. Aspirin “resistance” and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ 2008; 336: 195-8.

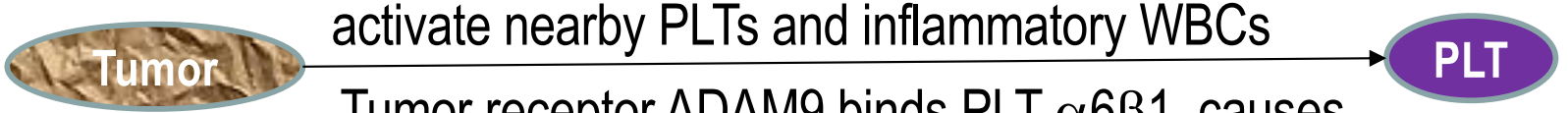
Platelet-Tumor Crosstalk



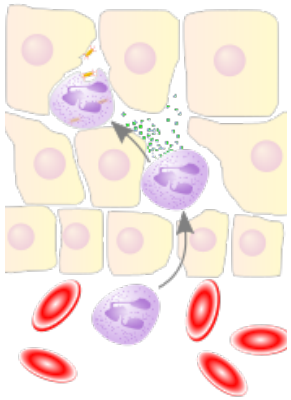
Xiaohong RL, Yousef GM, Ni H. Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. Blood 2018; 131:1777–99.

"Tumor-educated Platelets" (TEP) AKA Tumor Cell-induced Platelet Aggregation (TCIPA)

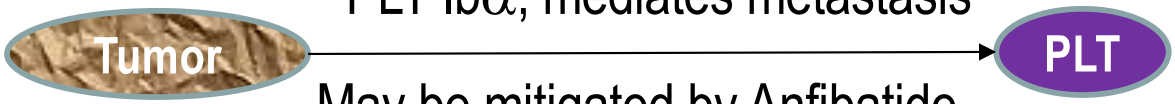
Tumor receptor αVβ3 binds PLT αIIbβ3 (IIb-IIIa) via fibrinogen, aggregates activate PLTs, generate fibrin, activate nearby PLTs and inflammatory WBCs



Tumor receptor ADAM9 binds PLT α6β1, causes tumor cell extravasation

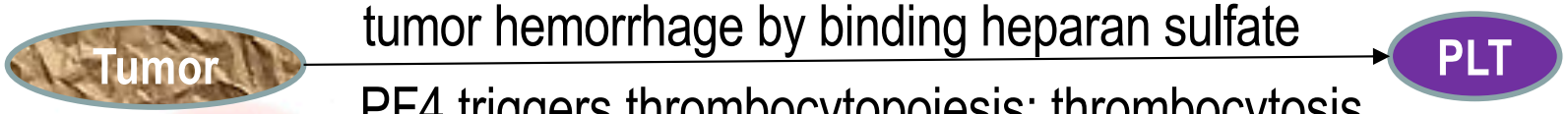


Tumors synthesize VWF, binds PLT Ibα, mediates metastasis



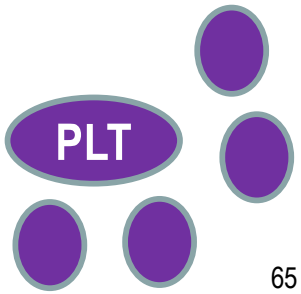
May be mitigated by Anfibatide, a venom anti-Ibα polypeptide

Activated PLTs secrete vascular endothelial GF, platelet-derived GF, trigger tumor angiogenesis. PF4 mitigates tumor hemorrhage by binding heparan sulfate



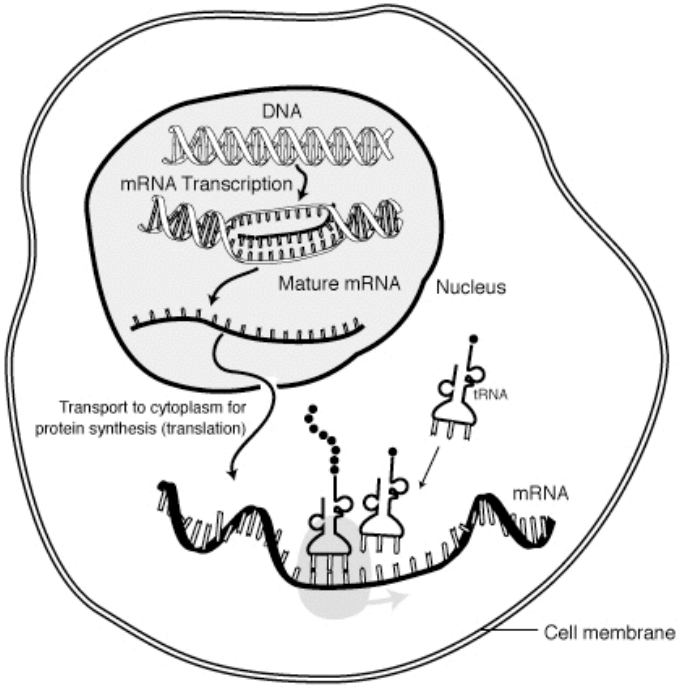
PF4 triggers thrombocytopoiesis; thrombocytosis predicts cancer risk, thrombocytopenia reduces risk and enhances chemotherapy.

Activated PLTs generate microparticles that stimulate lung tumors, also a lab tumor marker



"Tumor-educated Platelets" (TEP) Tumors Alter Platelet RNA

- PLTs gain tumor markers
- Platelet mRNA sequencing identifies cancer with 96% accuracy
  - Assay is simplified without nuclear DNA or mitochondria
- Sequencing IDs glioblastoma, colorectal, pancreatic, hepatobiliary, breast, and non-small-cell lung cancer with 71% accuracy
- Tumors can likewise "adopt" PLT RNA and markers to evade immunosurveillance.



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Platelets Support Tumor Growth and Metastasis

PLT transforming growth factor β (TGFβ) proliferates ovarian tumors

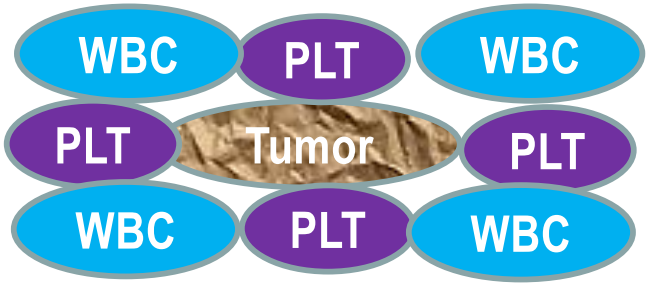
PLT → Tumor, Tumor, Tumor

PLTs transform leukemia cells to resist apoptosis

PLTs transform epithelial cells so they invade and seed nearby tissues

PLT → Tumor, Tumor

Hetero-aggregates of tumor cells, PLTs, and WBCs evade shear damage & immunosurveillance, support extravasation and seeding. Tumor cells "hitch a ride" on PLTs and WBCs to metastasize



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Aspirin Protects Against Cancer

- In 2016, US Preventive Service Task Force recommended 75–100 mg/d ASA for ages 50–69 to reduce risk of cardiovascular disease and colorectal cancer
- COX-2 is overexpressed in colorectal, breast, gastric, lung, pancreatic cancer, and melanoma. COX-2 expression is upregulated by nearby activated platelets.
- PGE2, produced in the eicosanoid synthesis pathway enhances tumor proliferation, angiogenesis, differentiation, inflammation, and immune escape.
- Slow-release 75 mg ASA sufficient to reduce incidence, metastasis, mortality, but…
- Doses up to 600 mg/d needed to suppress COX-2

Bibbins-Domingo K. Preventive Services Task Force. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer. US Preventive Services Task Force Statement. Ann Intern Med 2016; 53:409–30.

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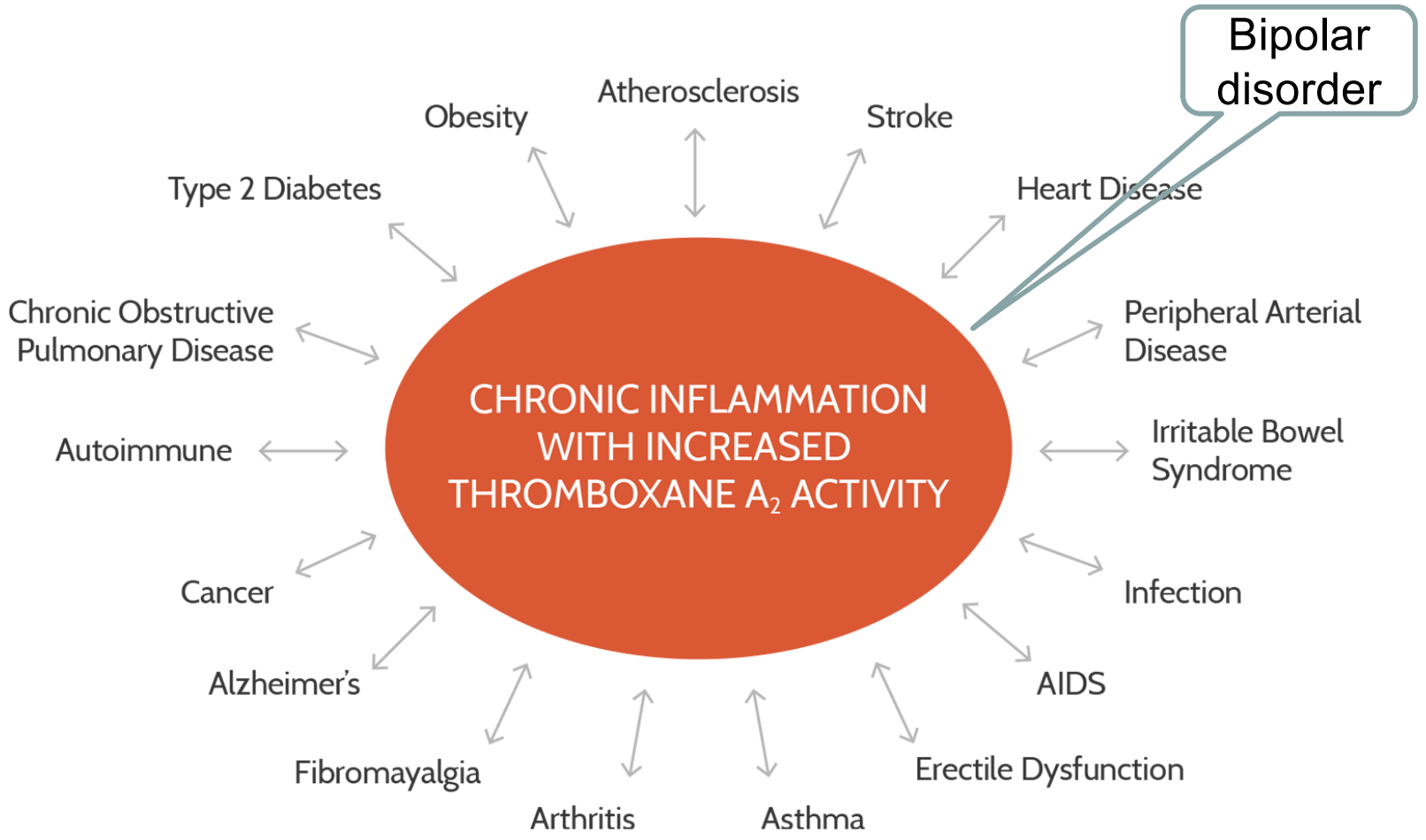
DISEASES OF CHRONIC INFLAMMATION



Savitz JB, Teague TK, Misaki M, et al. Treatment of bipolar depression with minocycline and/or aspirin: an adaptive, 2Å~2 double-blind, randomized, placebo controlled, phase IIA clinical trial. Translational Psychiatry2018;8

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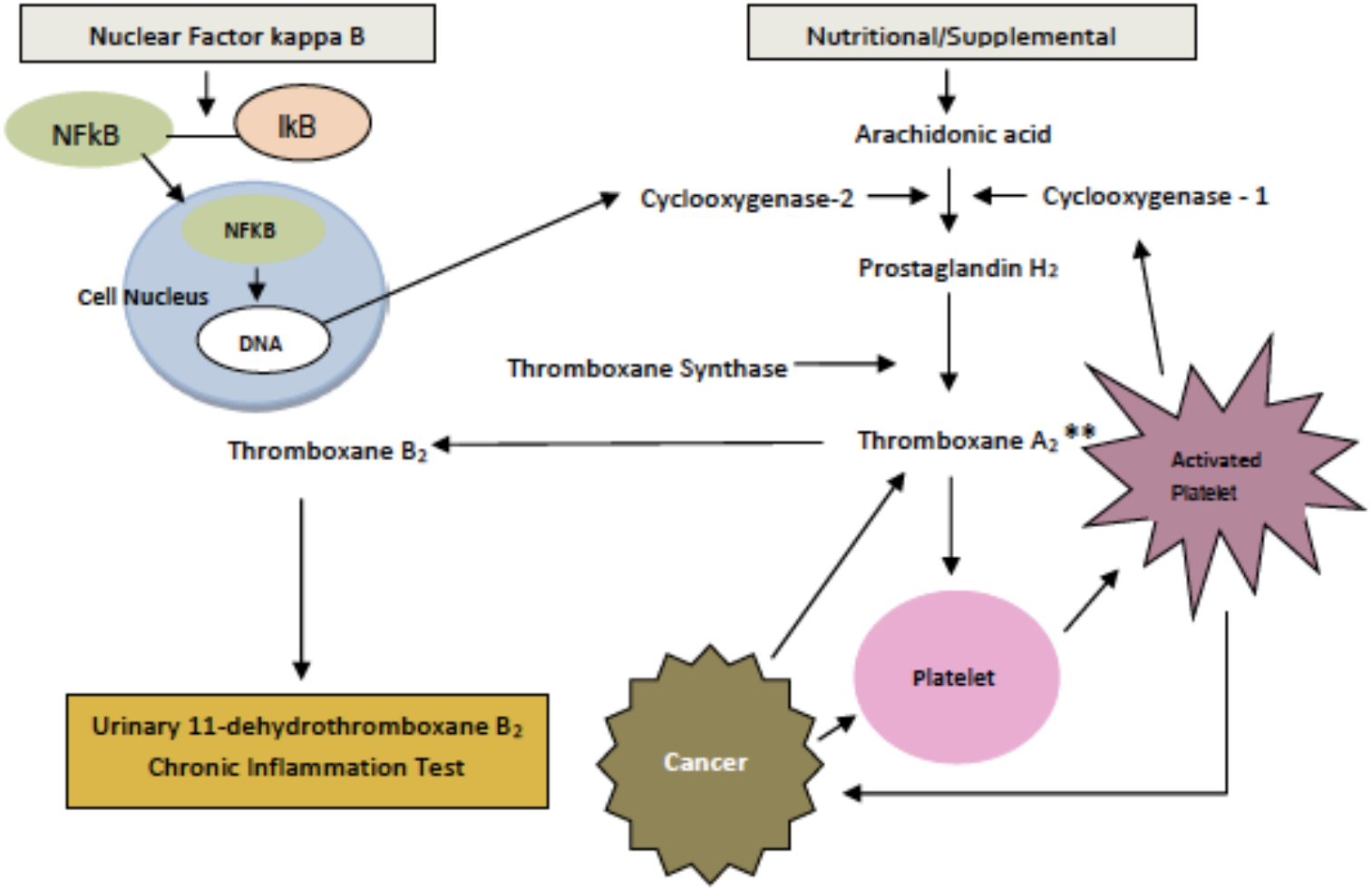
Inflammatory Markers Laboratory

Inflammation, activated platelets and thromboxane A2 generation in occurrence, progression and metastasis of cancer



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Activated Platelets and Cancer References

1. Battinelli EM, Markens BA, Kulenthirarajan RA, et al. Anticoagulation inhibits tumor cell–mediated release of platelet angiogenic proteins and diminishes platelet angiogenic response. Blood 2014; 123:101–12.
2. Karachaliou N, Pilotto S, Bria E, Rosell R. *Platelets and their role in cancer evolution and immune system*. Translational Lung Cancer Research. 2015.
3. Olsson AK, Cedervall J. The pro-inflammatory role of platelets in cancer. Platelets. 2018.
4. Li N. Platelets in cancer metastasis: To help the “villain” to do evil. International Journal of Cancer. 2015.
5. Mitrugno A, Sylman J, Ngo A, Pang J, et al. Aspirin therapy reduces the ability of platelets to promote colon and pancreatic cancer cell proliferation: Implications for the oncoprotein c-MYC. The American Physiological Society. 2017.
6. Li H, Lee M, Liu K, Wang T, et al. Inhibiting breast cancer by targeting the thromboxane A2 pathway. Nature Partner Journals. 2017.

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Activated Platelets and Cancer References

6. Ferroni P, Santilli F, Cavaliere F, Simeone P, et al. Oxidant stress as a major determinant of platelet activation in invasive breast cancer. International Journal of Cancer. 2016.
7. Li H, Liu K, Boardman L, Zhao Y, et al. Circulating prostaglandin biosynthesis in colorectal cancer and potential clinical significance. EBioMedicine. 2014.
8. Cho M, Noh K, Haemmerie M, Li D, et al. Role of ADP receptors on platelets in the growth of ovarian cancer. Blood. 2017.
9. Wojtukiewicz M, Hempel D, Sierko E, Tucker S, et al. Antiplatelet agents for cancer treatment: a real perspective or just an echo from the past? Cancer Metastasis Review. 2017.
10. Dovizio M, Alberti S, Guillem-Llobat P, Patrignani P. Role of platelets in inflammation and cancer: novel therapeutic strategies. Nordic Pharmacological Society. 2013
11. Lopez L, Guyer K, De La Torre I, Pitts K, et al. Platelet thromboxane (11-dehydro-Thromboxane B2) and aspirin response in patients with diabetes and coronary artery disease. World Journal of Diabetes. 2014.

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Thanks for Listening