Mice and Mothers: Progress in Understanding Pregnancy Complications in Patients with Lupus and Antiphospholipid Syndrome

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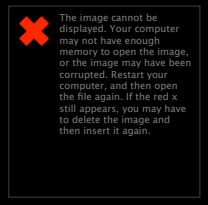

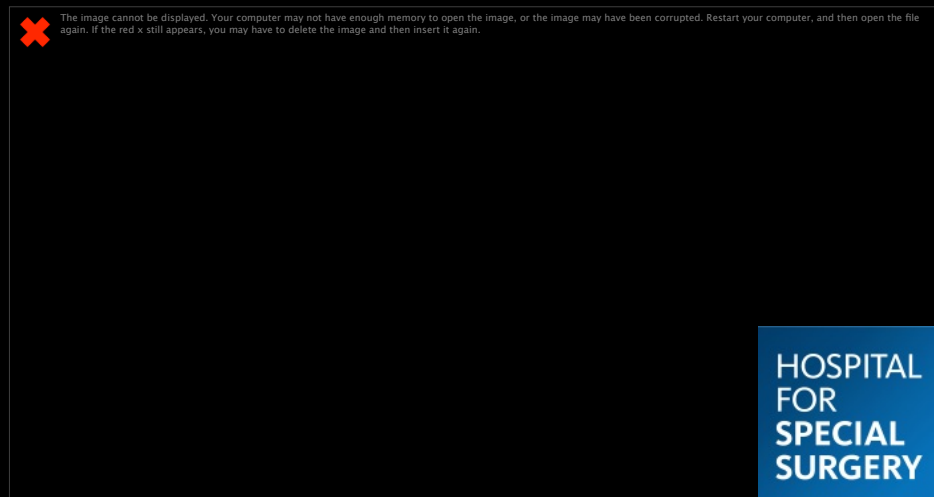

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Jane E. Salmon, MD

I DO have a financial interest in commercial products or services.

LIST TYPE OF RELATIONSHIP & COMPANY

Scientific Advisor: Alexion, BMS, Lilly, Kadmon, NovoNordisk

Investigator-initiated research grant: Kadmon

Obstetrical Outcomes in SLE and APS

- Identify clinical need
- Describe experimental models elucidating pathophysiology
- Translate discoveries to patients

Implications for prediction of risk for and pathogenesis of pregnancy complications in SLE and/or APS patients and potential targets for treatment

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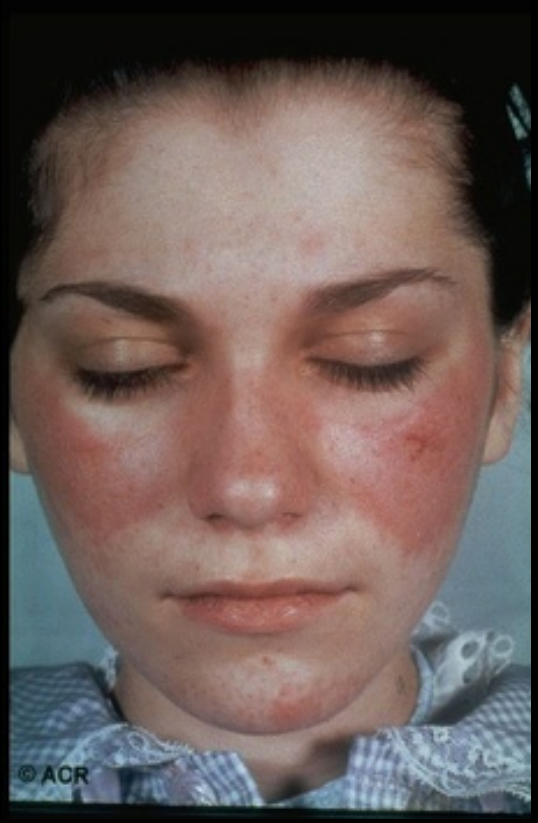


Lupus is a disease more frequent in women (9:1 female:male) with typical onset during the childbearing years.

Until recently, many patients with SLE were told that they should never become pregnant.

SLE: Multi-system Autoimmune Disease

Picasso, Pregnant Woman (1950) *Hirshhorn Museum and Sculpture Garden, Washington, DC*

Pregnancy Complications in SLE

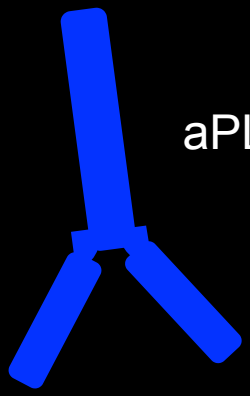
Hypertension

Preeclampsia

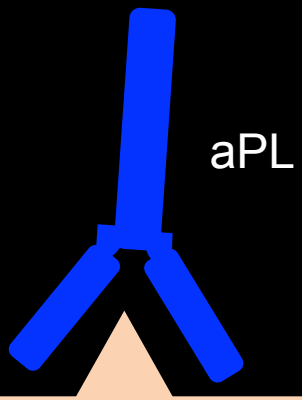
Placental insufficiency
Fetal growth restriction
Pregnancy loss

The presence of antiphospholipid antibodies (aPL) is the strongest predictor of pregnancy loss and other complications.

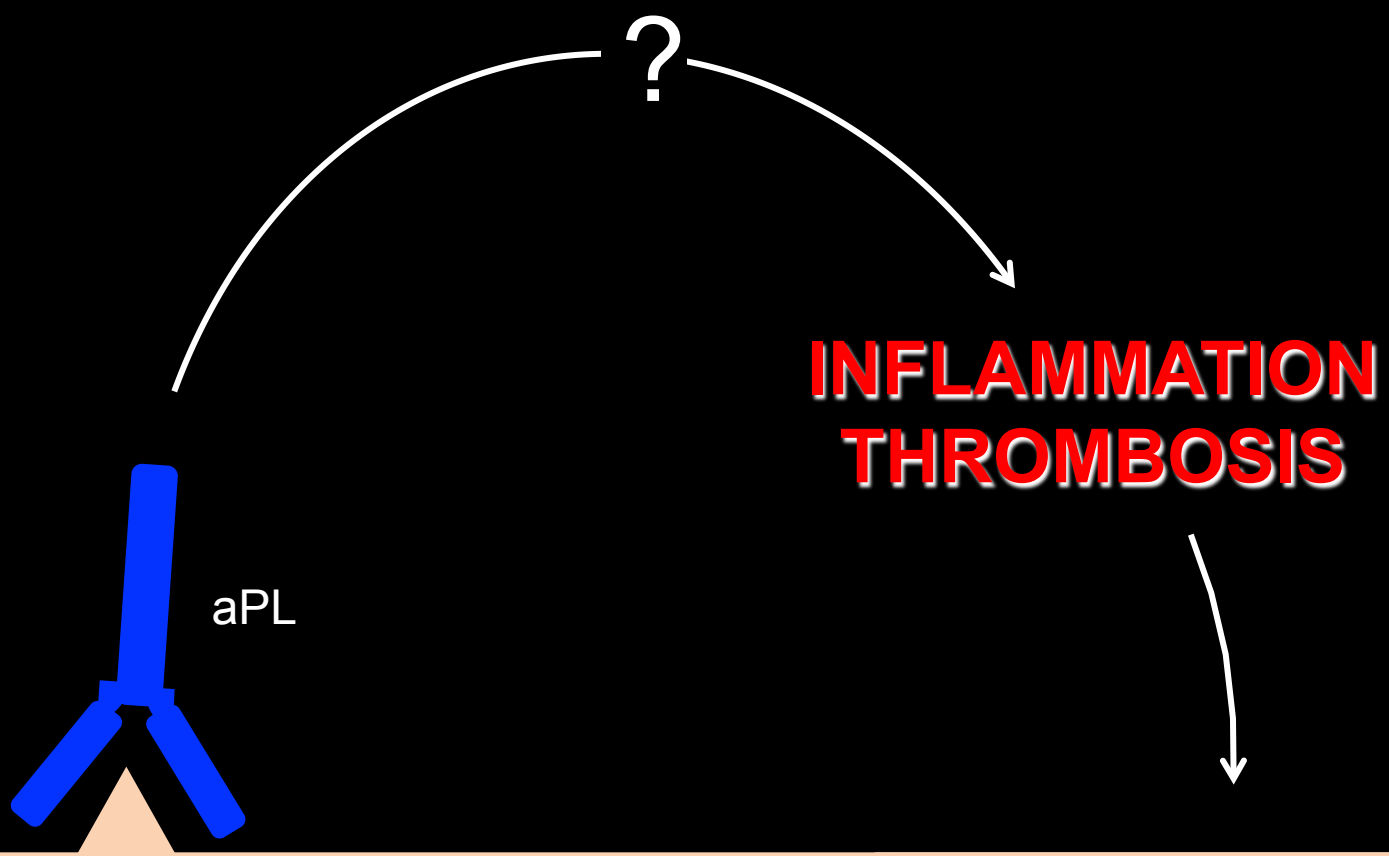
Picasso, Pregnant Woman (1950) *Hirshhorn Museum and Sculpture Garden, Washington, DC*



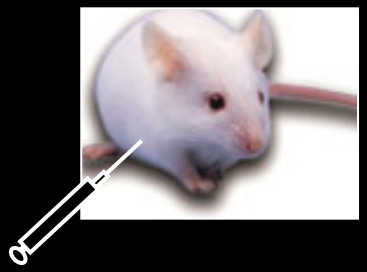
Placental cell surface



Placental cell surface


?
INFLAMMATION
THROMBOSIS
aPL
Placental cell surface
FETAL INJURY

METHODS : Murine Pregnancy Model



Passive transfer of human IgG (IP)

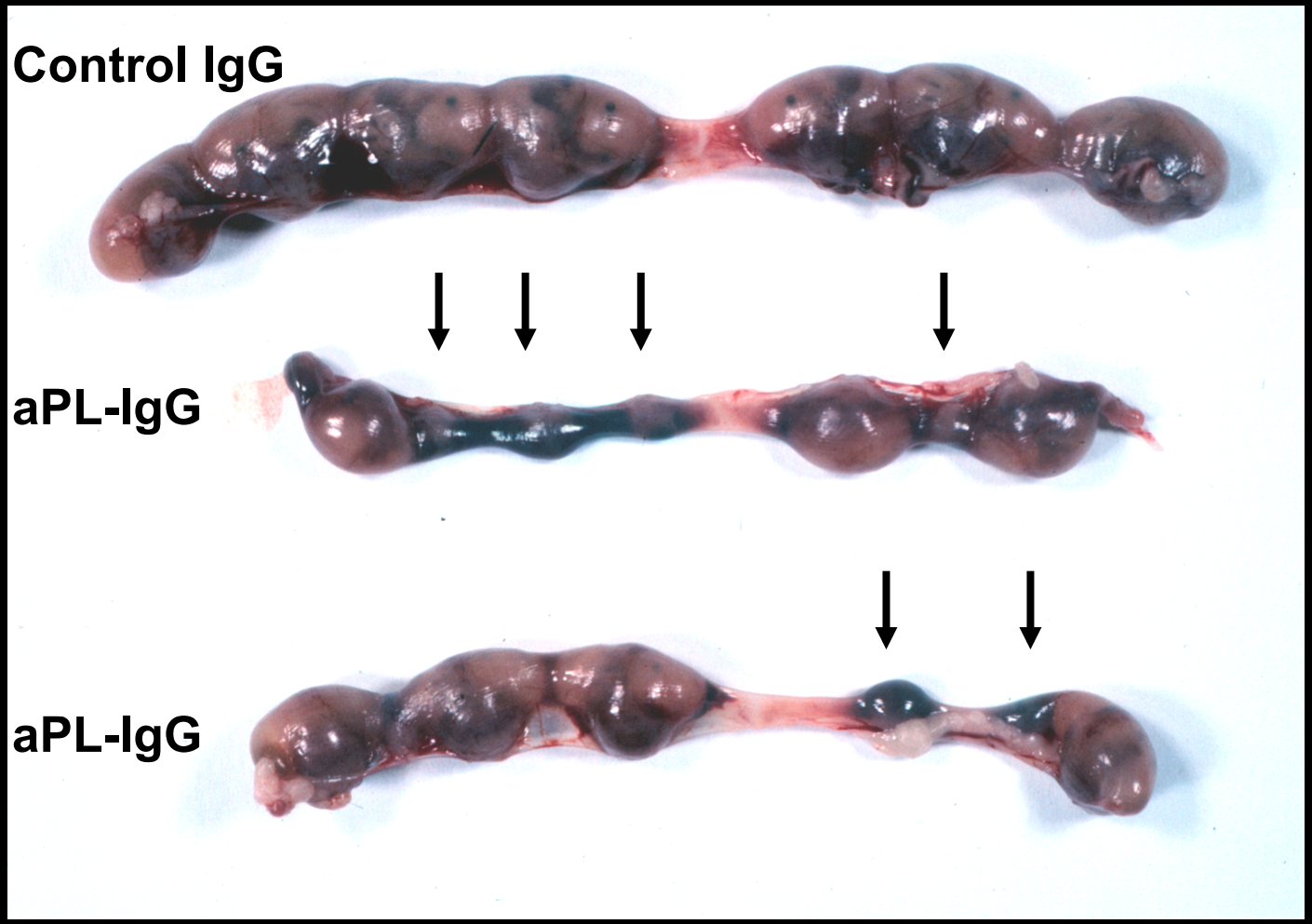
aPL-IgG or control IgG

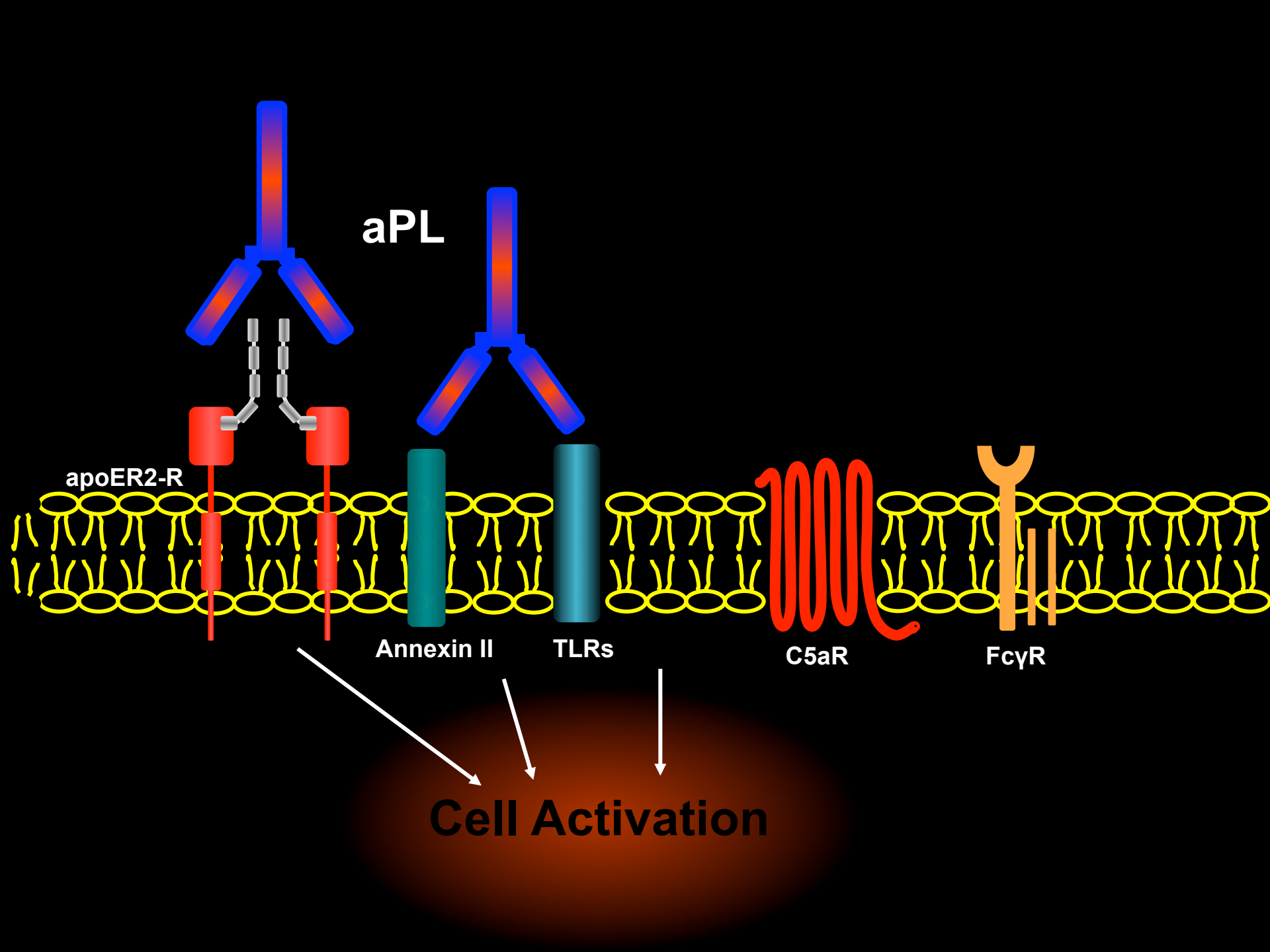
pregnancy

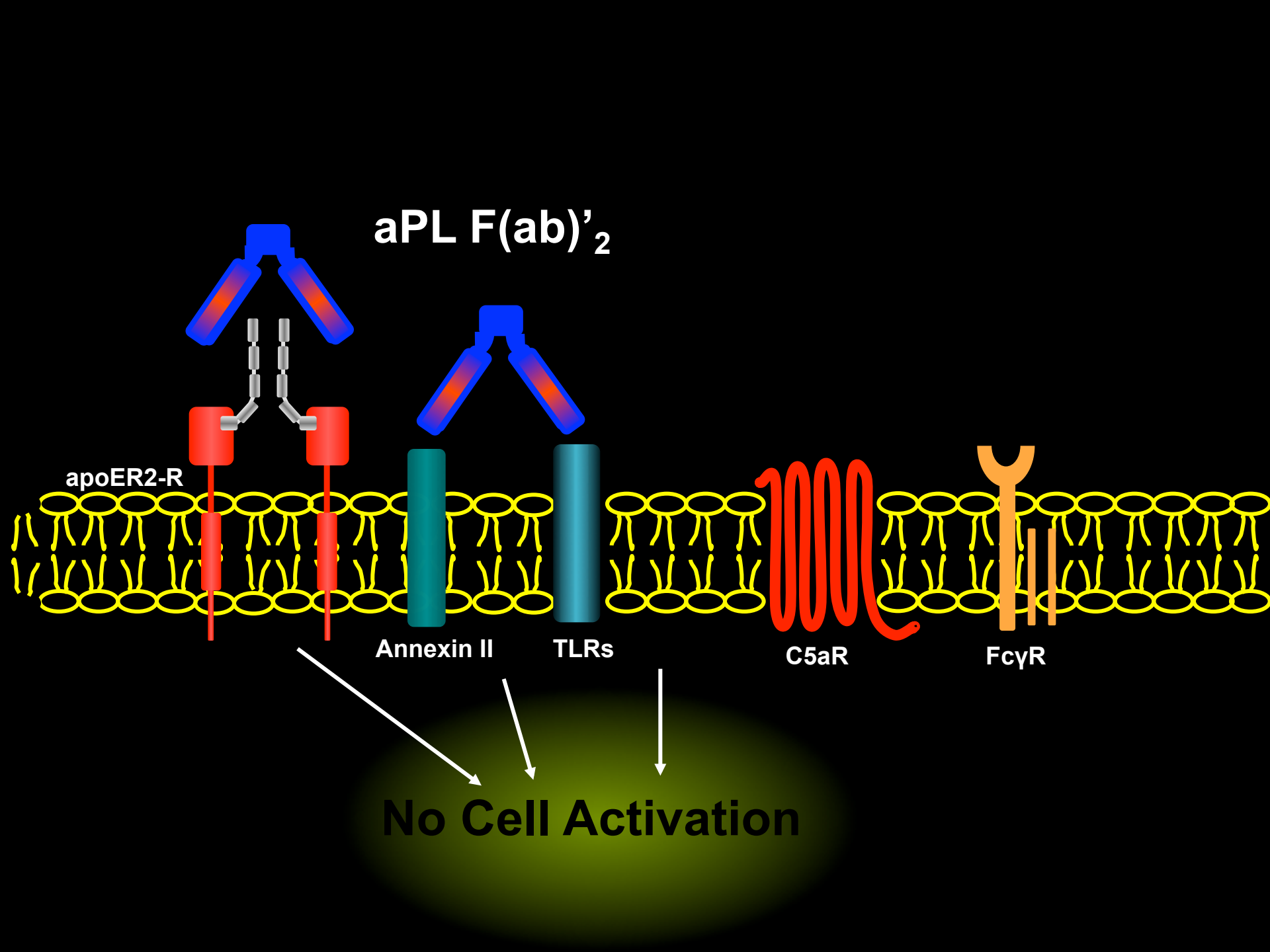
Day 0 | day 8 | day 10 | day 12 | day 15

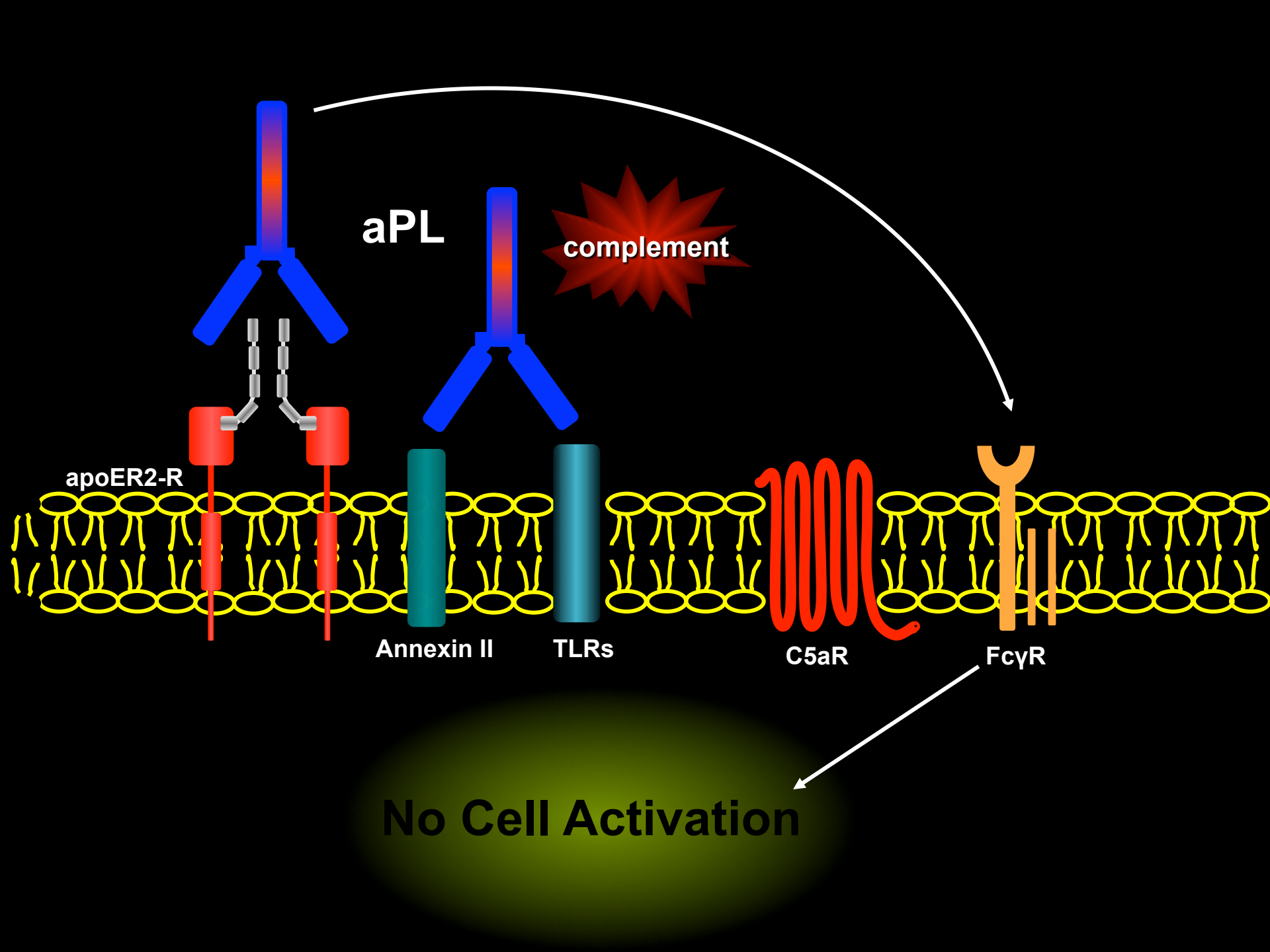
Dissection of uteri

Passive transfer of aPL antibodies in pregnant mice causes fetal loss and growth restriction

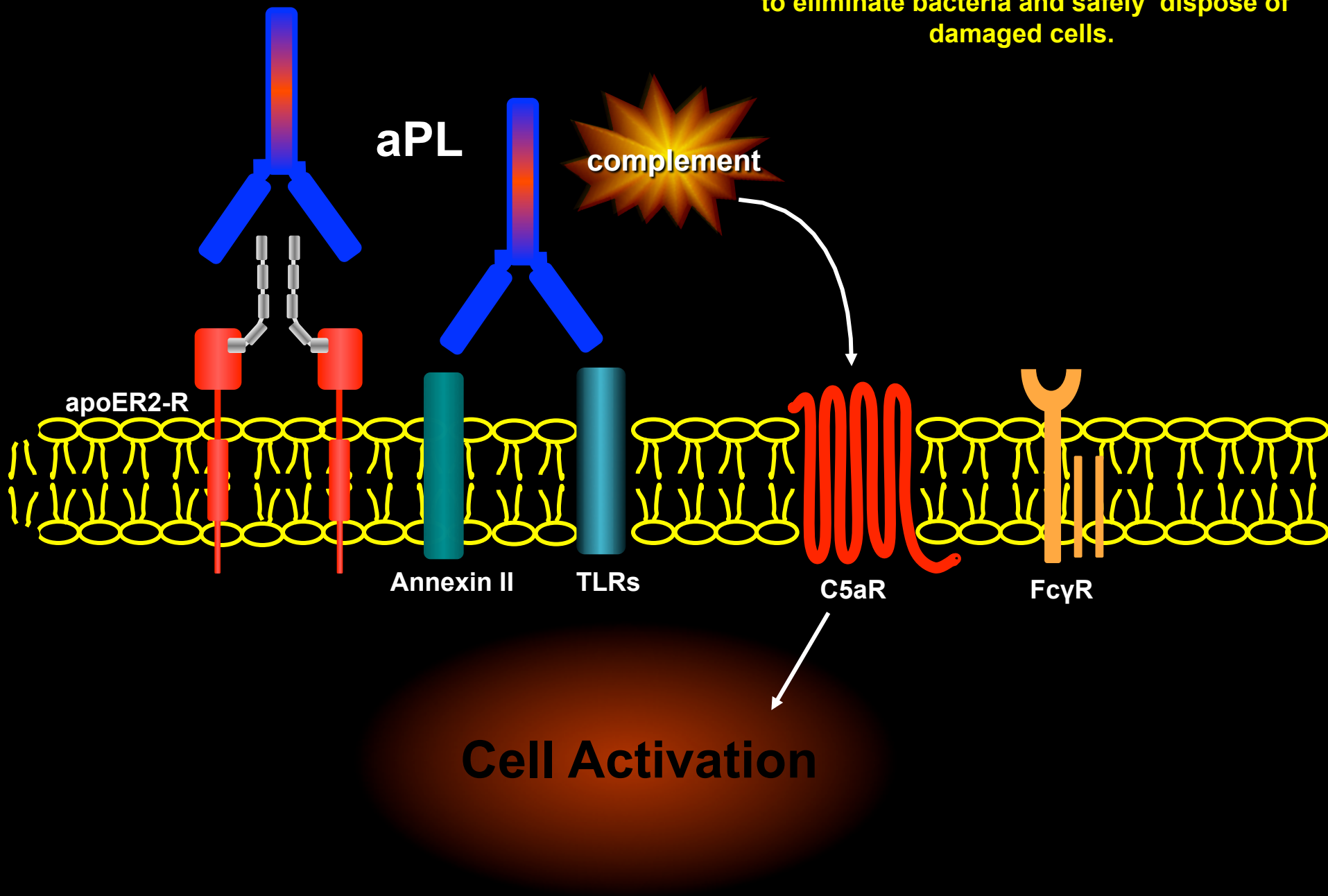


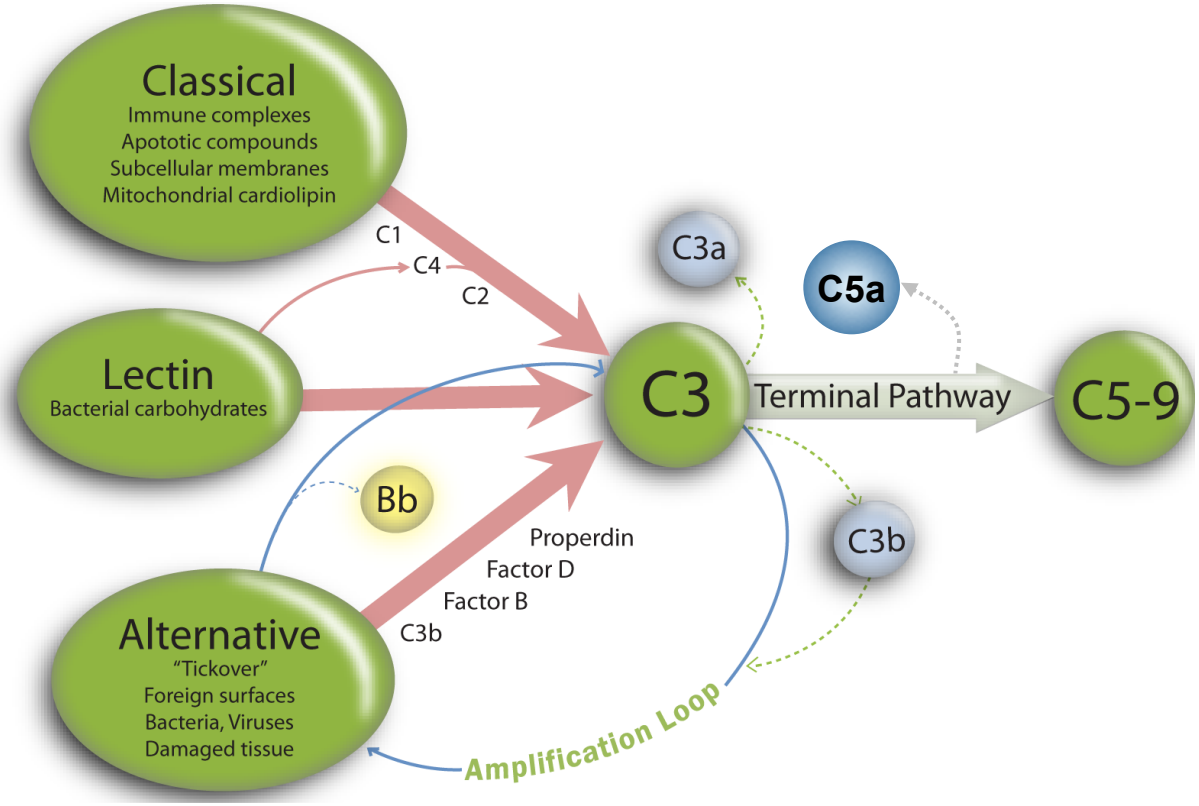

aPL
apoER2-R
Annexin II
TLRs
C5aR
FcγR
Cell Activation


aPL F(ab)'2
apoER2-R
Annexin II
TLRs
C5aR
FcγR
No Cell Activation

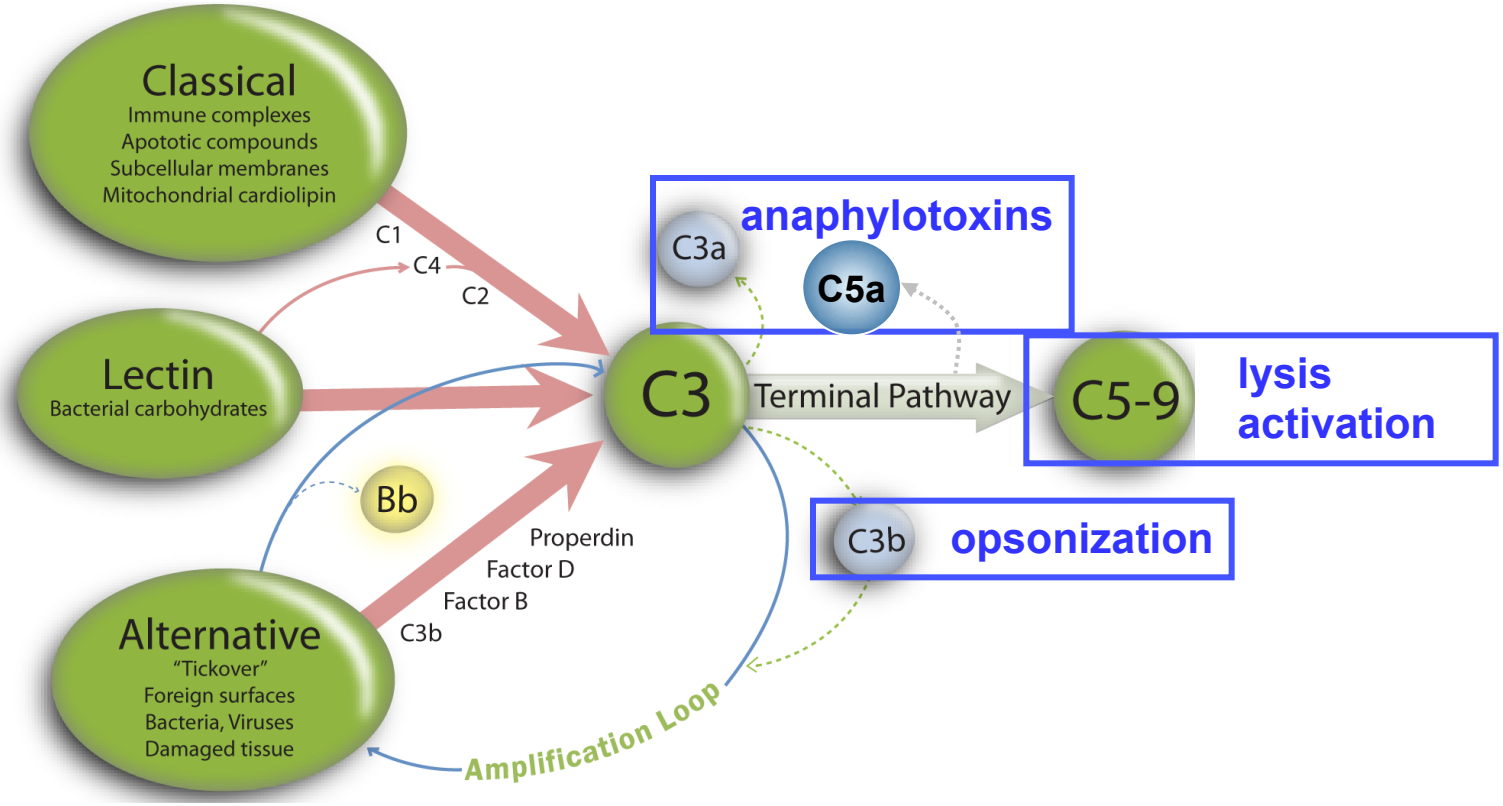

aPL
complement
apoER2-R
Annexin II
TLRs
C5aR
FcγR
No Cell Activation

Complement system is a family of proteins in the blood and on cells that work together to eliminate bacteria and safely dispose of damaged cells.

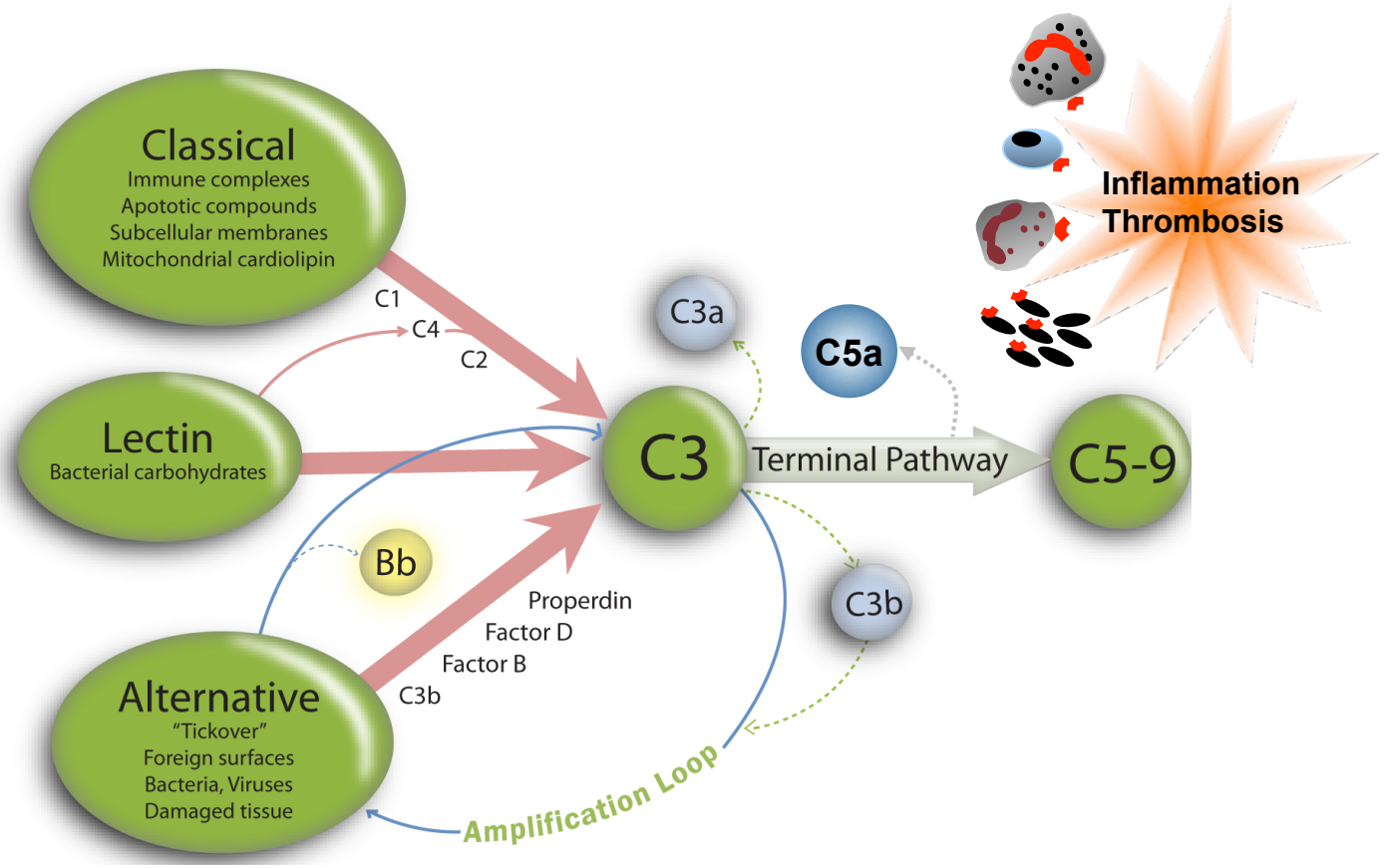




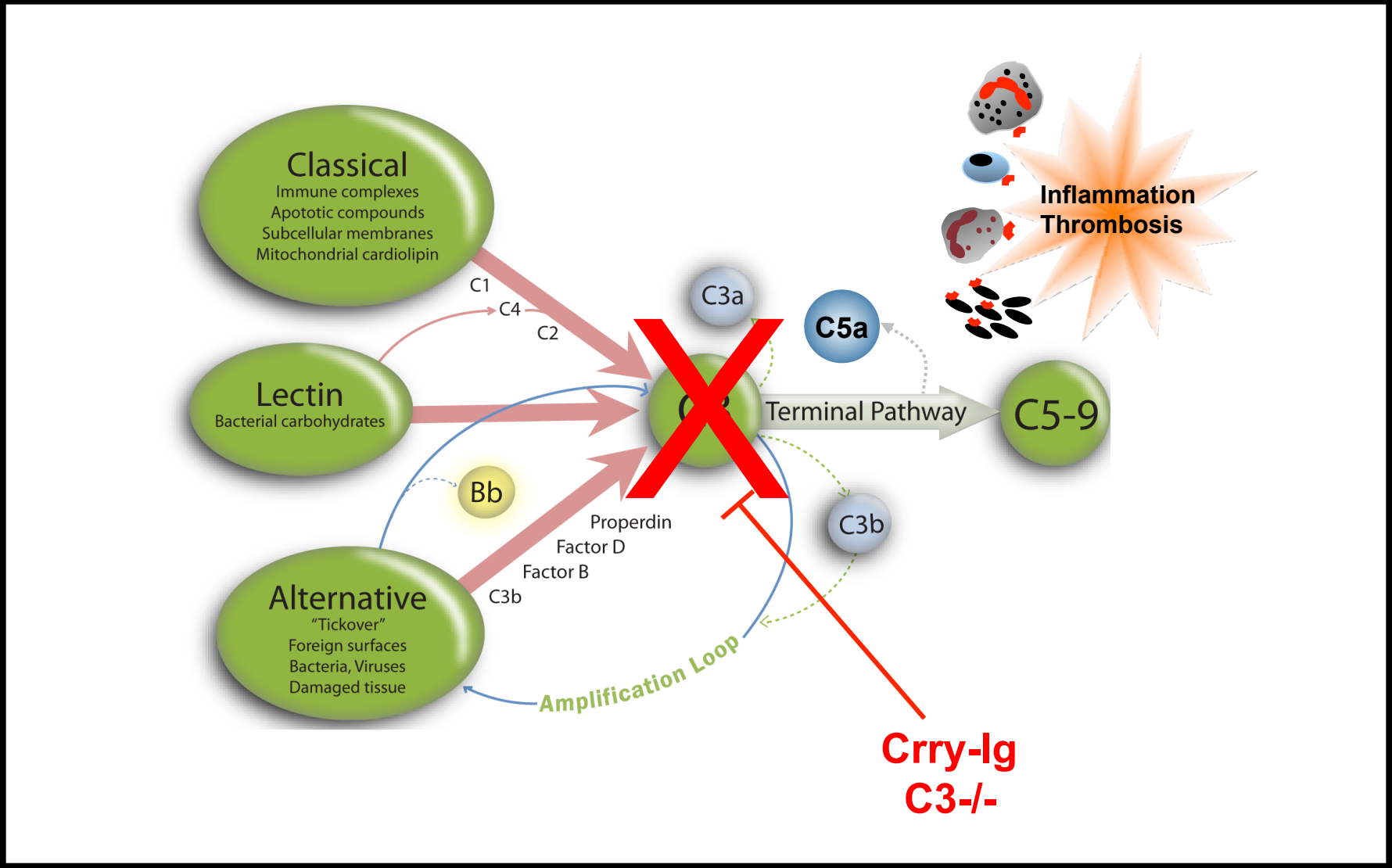
Lynch et al. *Obstet Gynecol* 2011



Lynch et al. *Obstet Gynecol* 2011

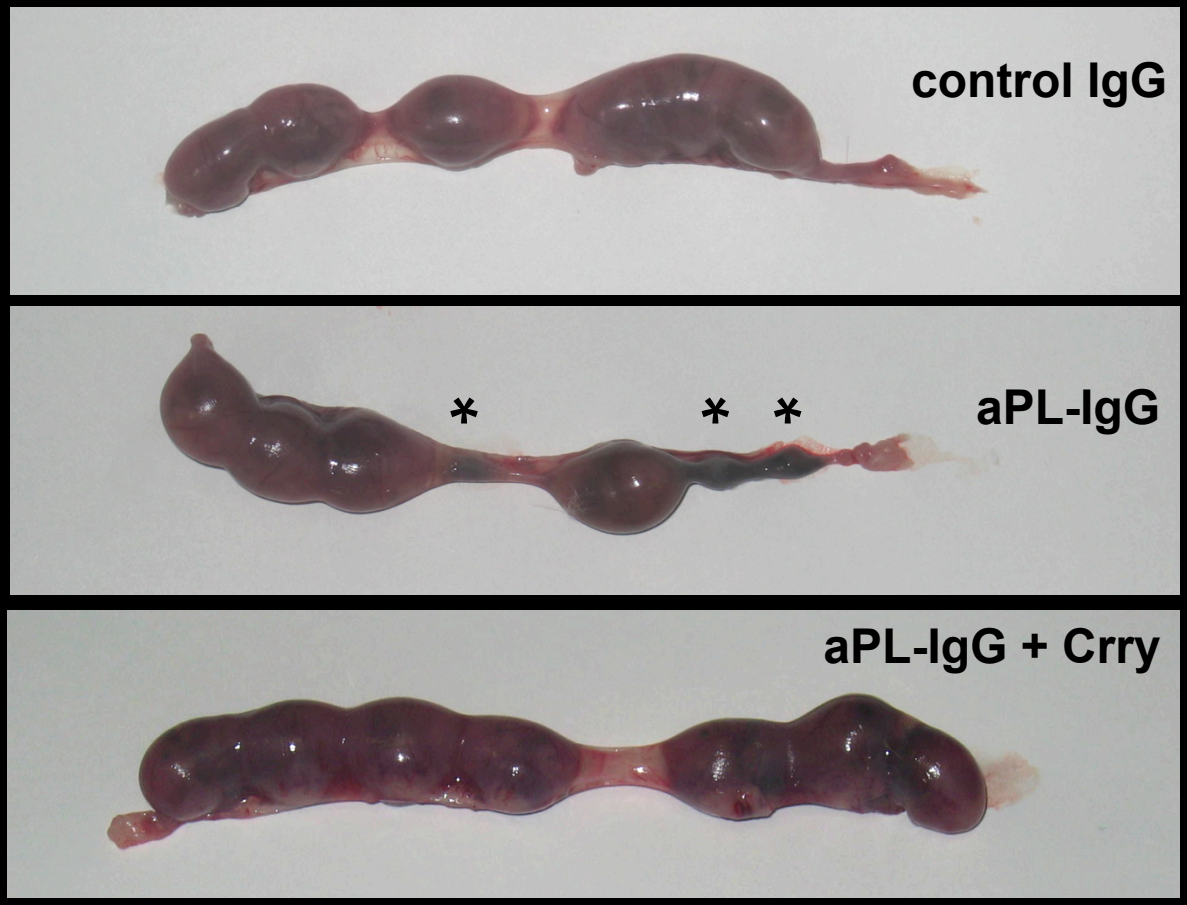


Lynch et al. *Obstet Gynecol* 2011



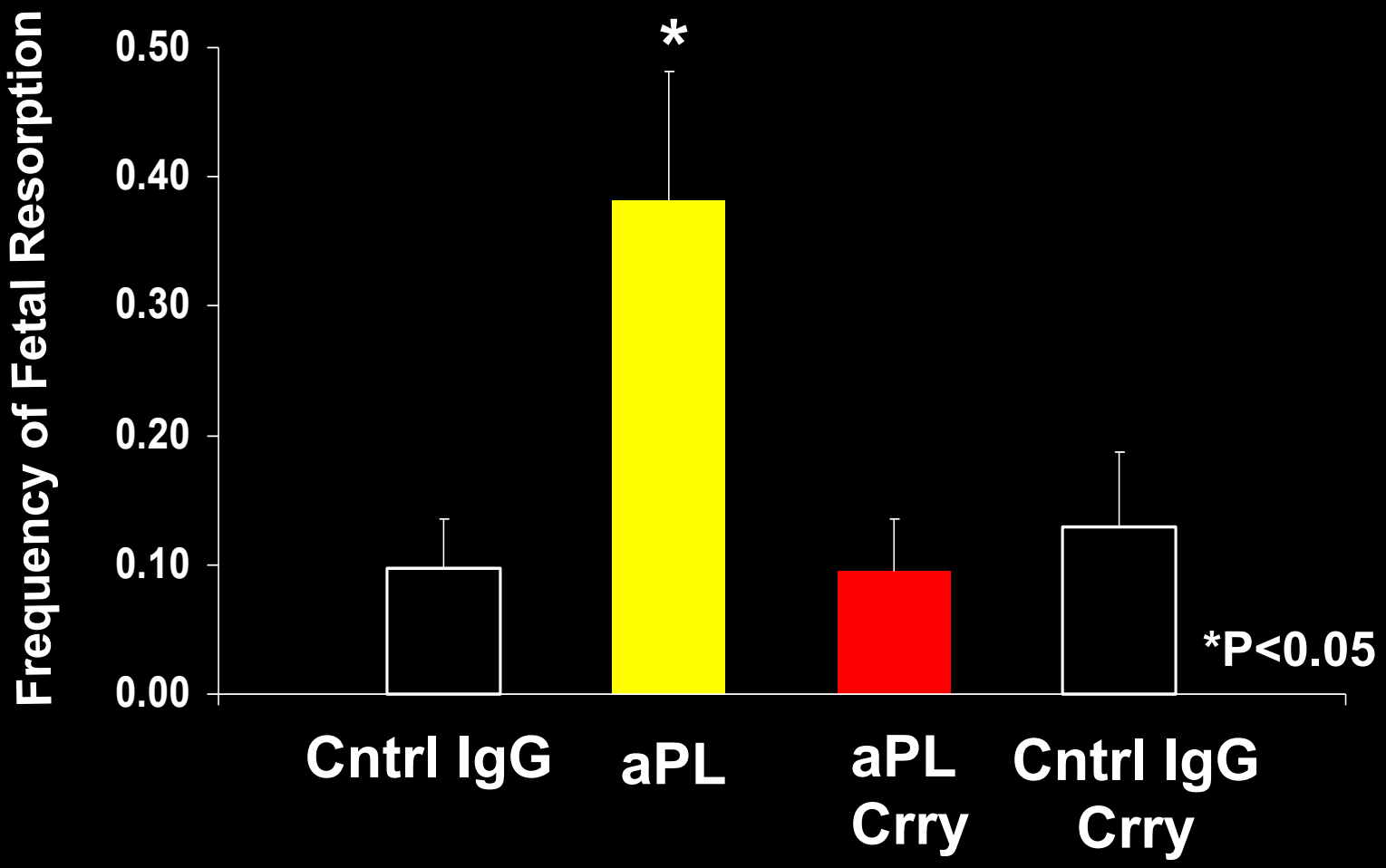
Lynch et al. *Obstet Gynecol* 2011

Blockade of the complement cascade with Crry protects from aPL antibody-induced pregnancy loss



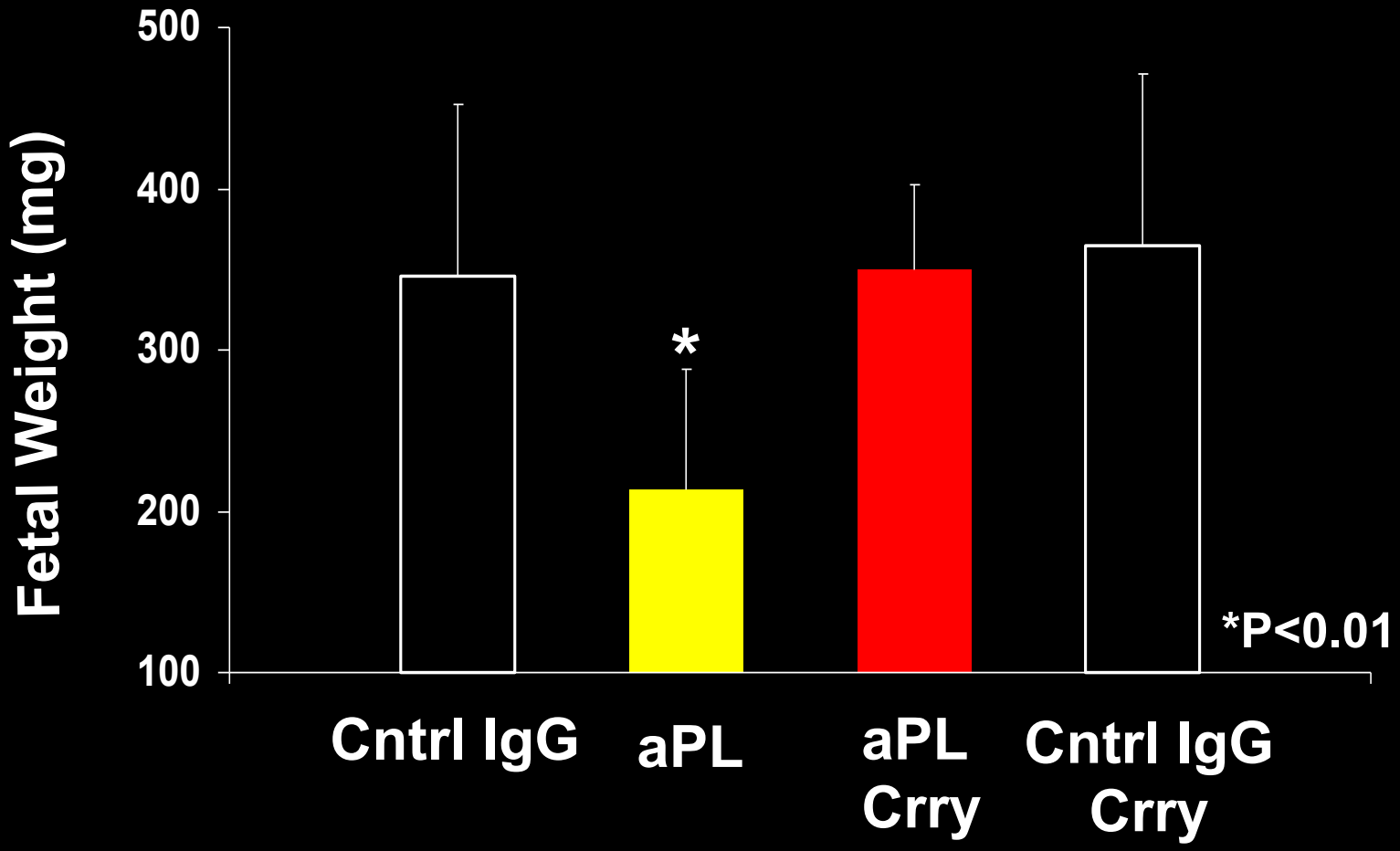
Holers et al. J Exp Med 195:211, 2002

aPL-induced fetal resorption is prevented by inhibiting activation of complement



Holers et al. J Exp Med 195:211, 2002

aPL-induced fetal growth restriction is prevented by inhibiting activation of complement



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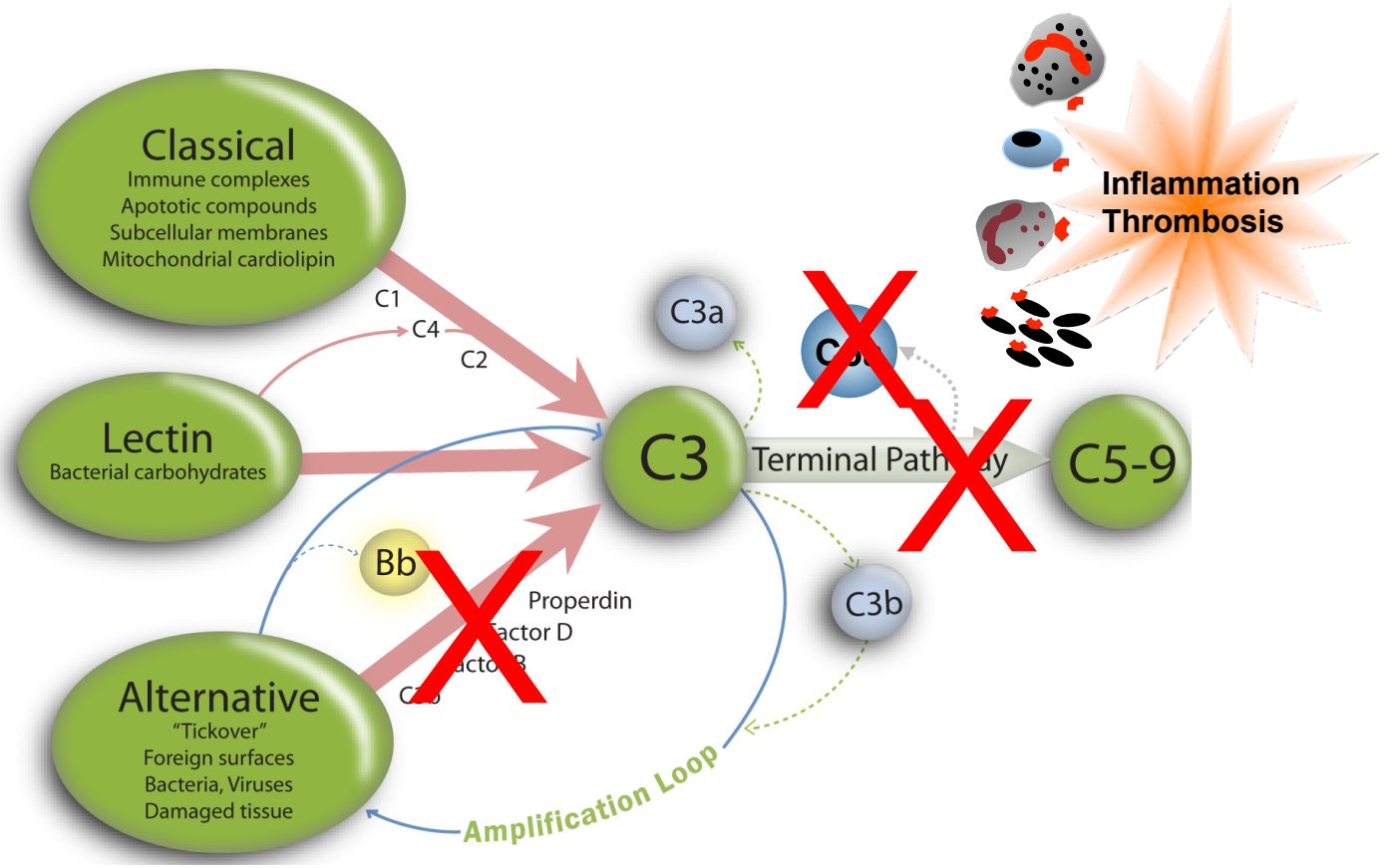

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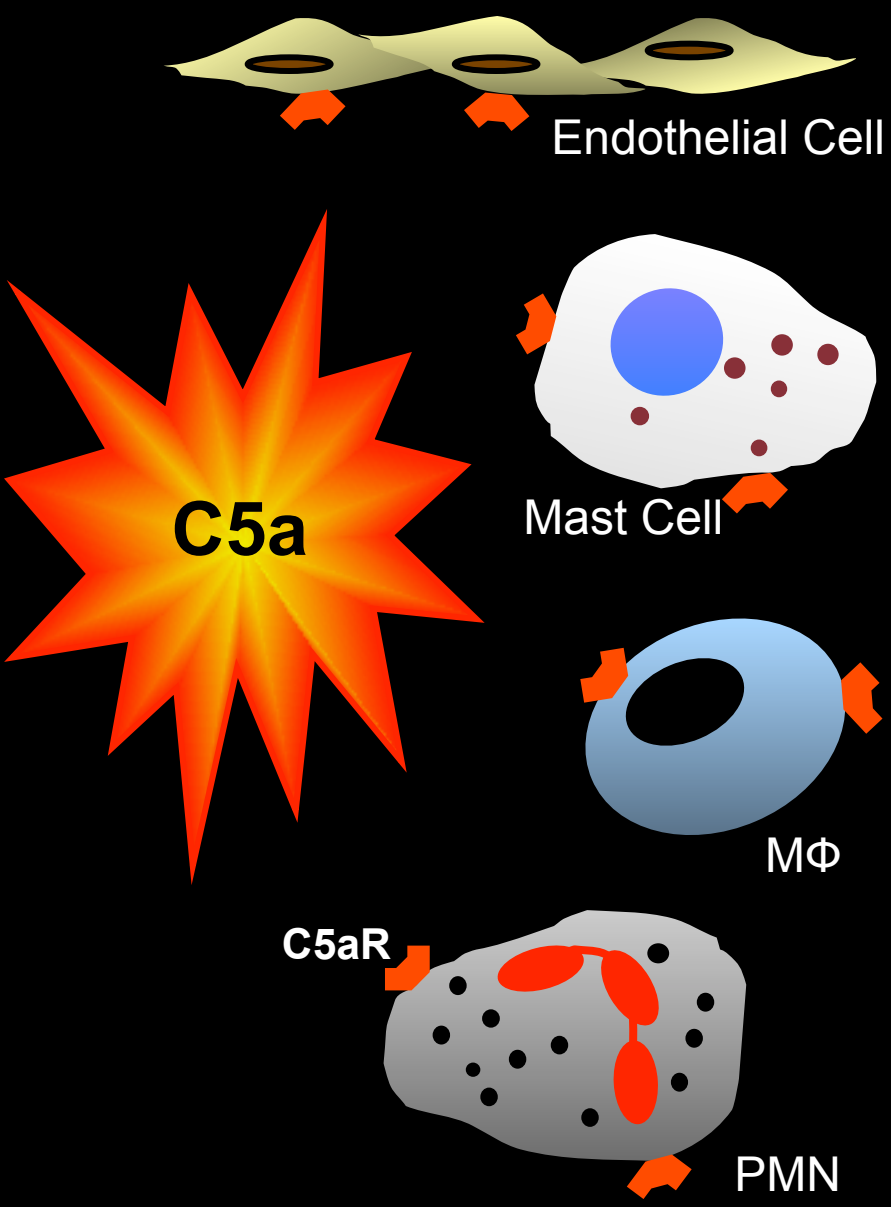


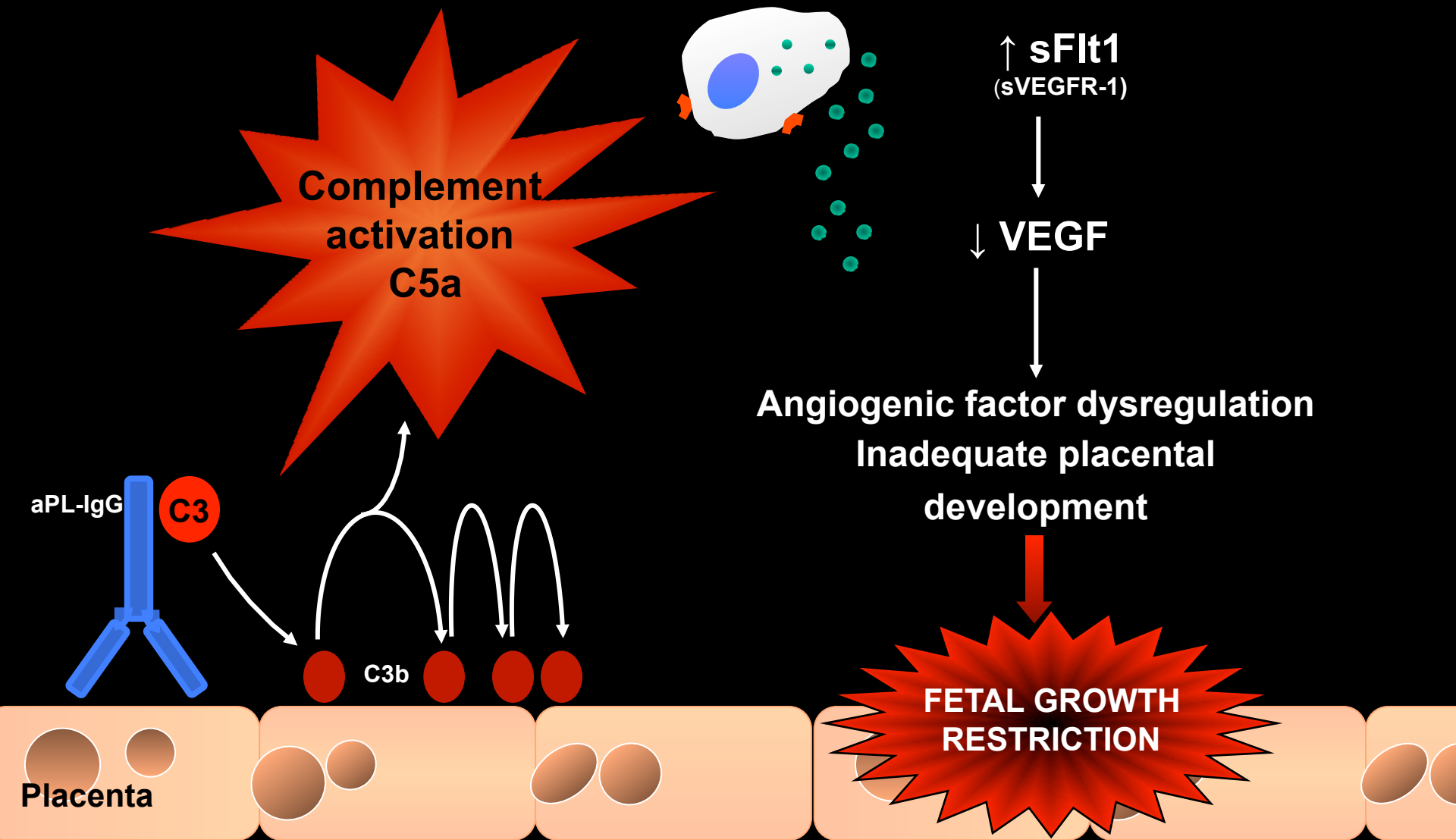
↑ β-integrins, P-selectin, Tissue factor (TF)
Angiogenic factors

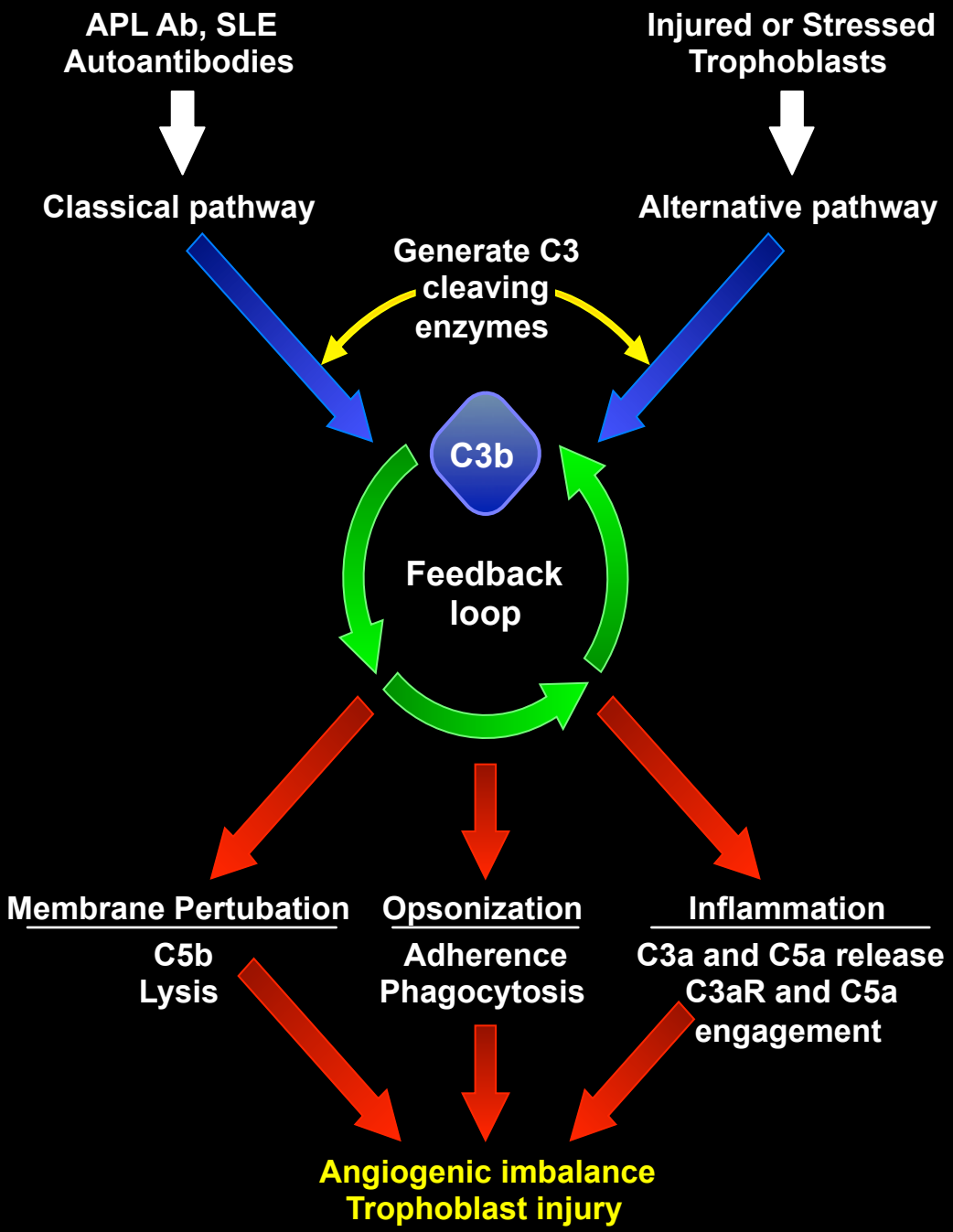
Chemokines and cytokines (TNF-α, IL-4, IL-13, IL-5, MIP- α)
Histamine, proteases, and lipid mediators

Oxidants, proteases and TF
Cytokines and chemokines (IL-1, IL-6, TNF-α, IL-8), and lipid mediators
Angiogenic factors

Generation of reactive oxidants, proteases
Cytokines (TNF-α), chemokines and lipid mediators, Complement components
Angiogenic factors

Complement activation causes angiogenic factor dysregulation





Salmon et al. PLoS Med, 2011

How can we predict risk?

The PROMISSE Study

Predictors of P**R**egnancy **O**utcome: Bio**M**arkers **I**n Antiphospholipid **S**yndrome and **S**ystemic Lupus **E**rythematosus

A prospective multicenter observational study to identify markers that predict poor pregnancy outcome in aPL and/or SLE patients

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PROMISSE Investigators

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Virginia Pascual (Baylor Immunol Institute, Dallas)

Anne Lynch (U Colorado, Denver)

Subjects

- Pregnant women ≤12 weeks' gestation grouped according to the presence or absence of SLE and/or APL antibodies

  SLE+ APL+
  SLE+ APL-
  SLE- APL+
  SLE- APL-

SLE+ is defined as ≥4 ACR criteria for SLE

APL+ is defined as at least one of following documented twice 6 weeks apart in a core lab: ACL (IgG or IgM ≥ 40 units), anti-β2GPI (IgG or IgM ≥ 40 units) or Lupus anticoagulant

Controls (APL-/SLE-) must have had ≥1 normal pregnancy and no history of fetal death at >10 wks or >1 miscarriage at <10 wks gestation

- Age 18-45 years and able to give informed consent

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Subjects

Exclusion Criteria

- Prednisone > 20 mg/day
- Renal disease (proteinuria >1000 mg/24 hr; serum Cr >1.2 mg/dl; RBC casts)
- Diabetes mellitus (Type I and Type II) antedating pregnancy
- Hypertension (blood pressure ≥140/90 mmHg)
- Multi-fetal pregnancy

Study Outcomes

The study outcome was a composite endpoint defined as the occurrence of one or more of the following events:

- Fetal death occurring after 12 weeks' gestation and not explained by chromosomal abnormalities, anatomic malformations, or congenital infections
- Neonatal death prior to hospital discharge and due to complications of prematurity and/or placental insufficiency
- Indicated preterm delivery prior to 36 weeks' gestation because of gestational hypertension, preeclampsia or placental insufficiency
- Small for gestational age (SGA) <5th %ile in the absence of anatomical or chromosomal abnormalities
- Preeclampsia defined as new onset of elevated blood pressure (≥140/90 mm Hg) AND proteinuria of 300 mg in a 24hr collection or ≥1+ on dipstick

Patient Assessments

Patients were seen monthly and 3 months post-partum:

- Physical examination and obstetric ultrasound
- Laboratory tests and urinalysis

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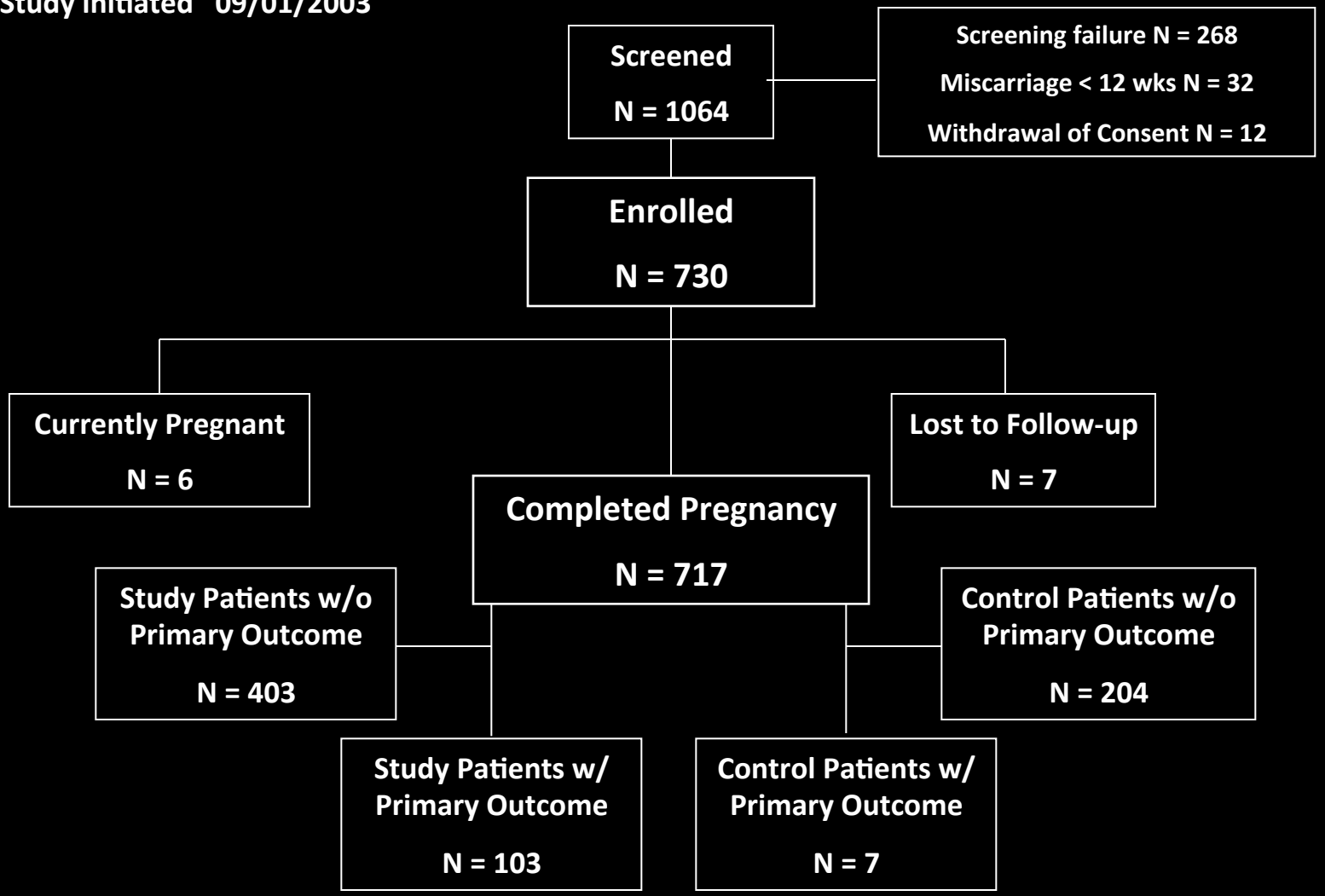
Study Status

Study initiated 09/01/2003



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Enrollment of PROMISSE Patients

	Number of Subjects Who Completed Pregnancy
SLE+ / APL+	60
SLE+ / APL-	338
SLE- / APL+	105
SLE+ and/or aPL+	503
SLE- / aPL-	204

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Prevalence of Outcomes in PROMISSE Patients

	Number of Subjects Who Completed Pregnancy	Primary Outcome(s) Among Completers
SLE+ / APL+	60	37%
SLE+ / APL-	338	16%
SLE- / APL+	105	26%
SLE+ and/or aPL+	503	20%
SLE- / aPL-	204	3%

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Prevalence of Outcomes in PROMISSE Patients

	Number of Subjects Who Completed Pregnancy	Primary Outcome(s) Among Completers	Neonatal Deaths	Fetal Deaths	SGA (<5th %ile)	Pre-Term Delivery
SLE+ / APL+	60	37%	2%	13%	18%	20%
SLE+ / APL-	338	16%	1%	2%	8%	7%
SLE- / APL+	105	26%	0%	11%	10%	13%
SLE+ and/or aPL+	503	20%	1%	5%	10%	10%
SLE- / aPL-	204	3%	0%	1%	3%	1%

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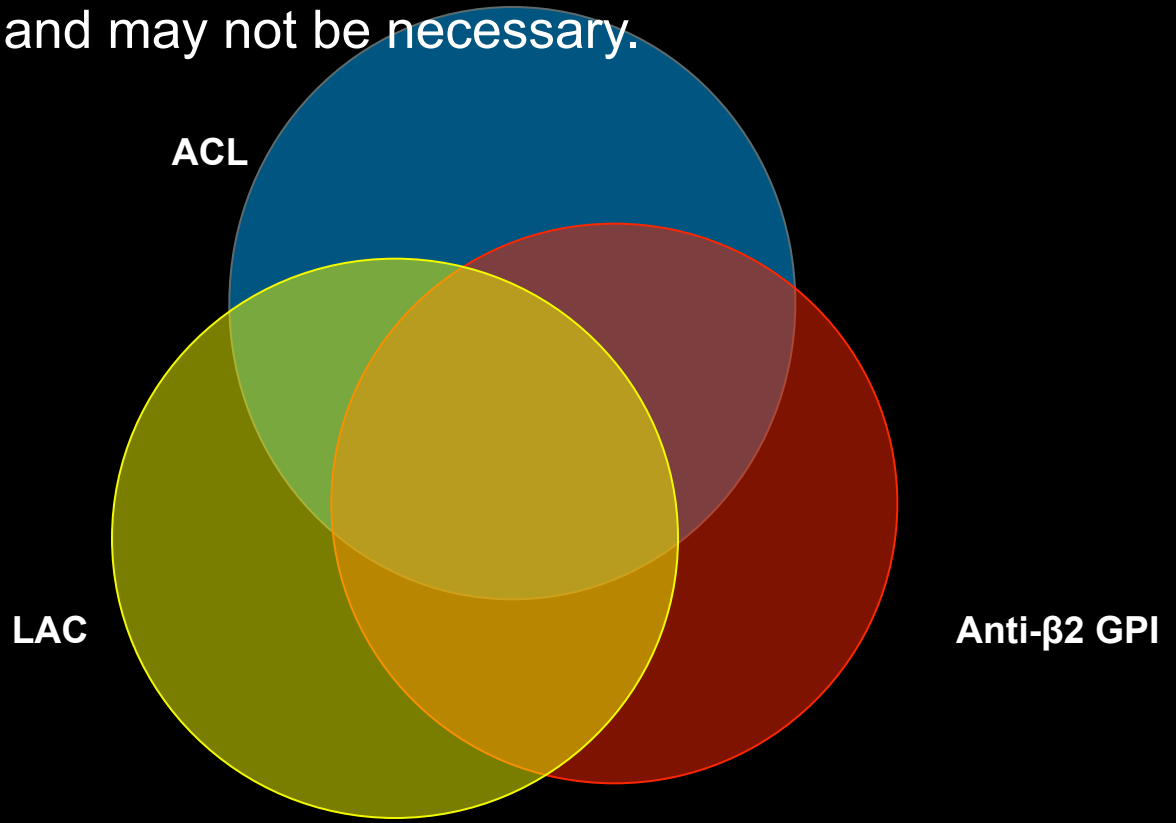
PROMISSE Discovery 1

Historical:

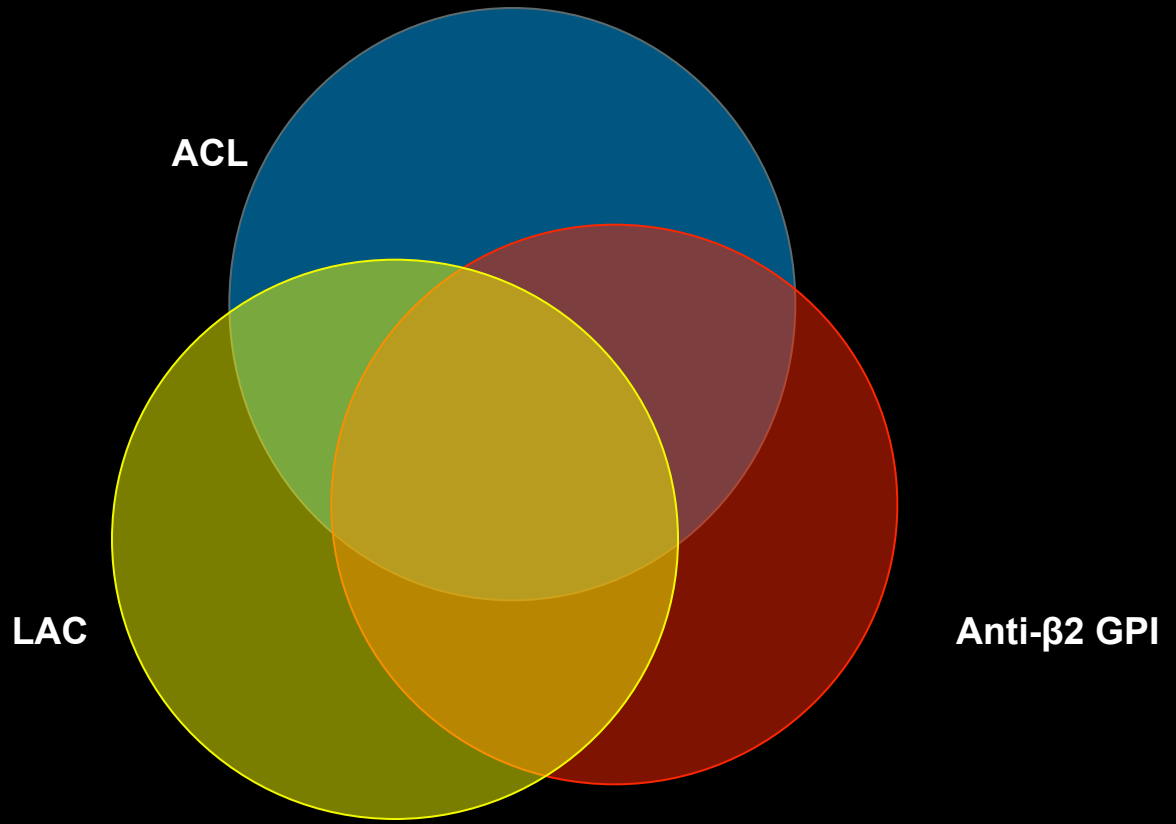
- Patients with all types of aPL antibodies were considered at similar risk for pregnancy complications.
- Patients with aPL antibodies are often treated with anticoagulation during pregnancy, but these drugs have side effects, are painful to administer, and may not be necessary.



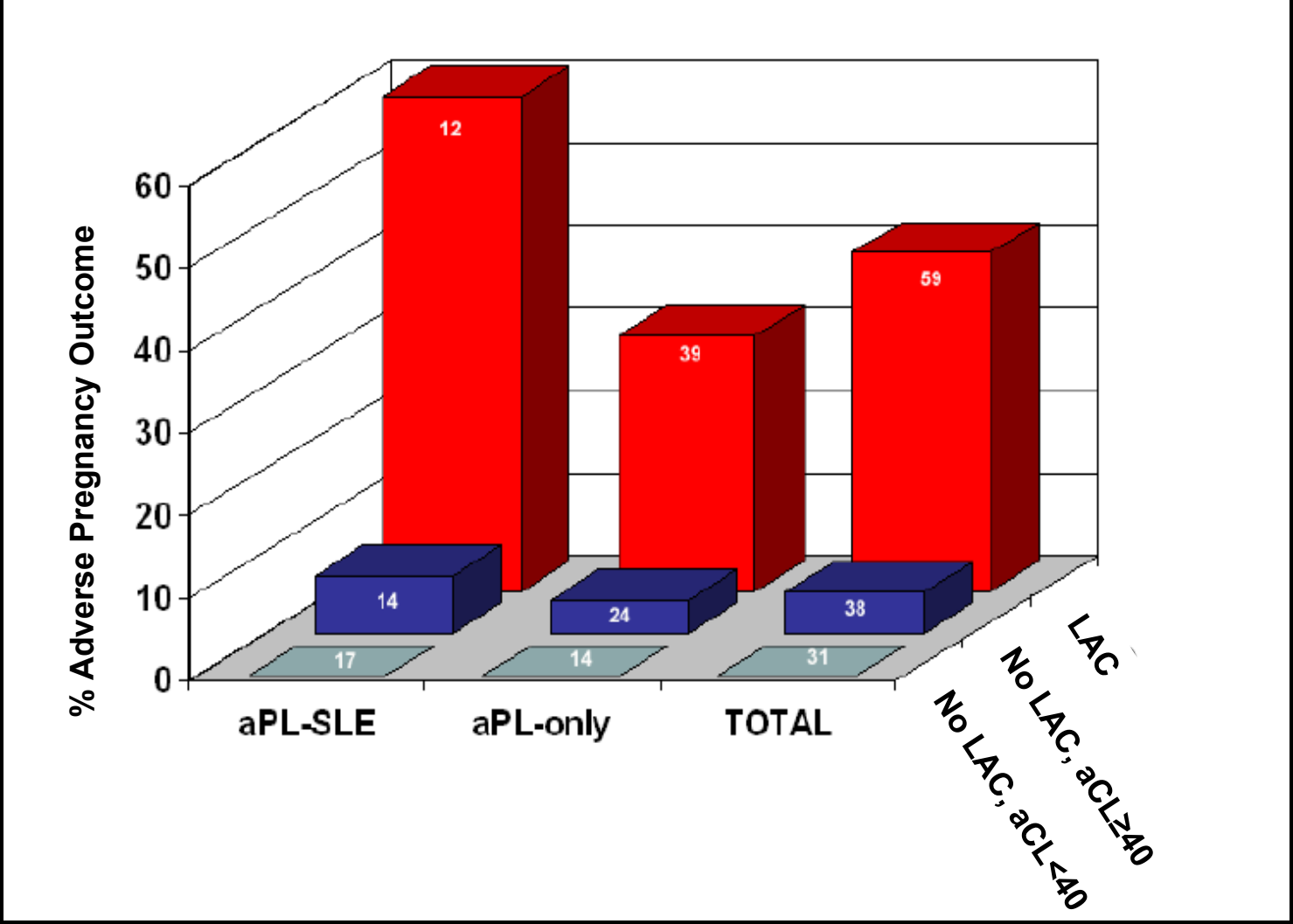
PROMISSE Discovery 1

Observation:

- Lupus anticoagulant (LAC) is the most powerful predictor of poor pregnancy outcomes in aPL-positive patients.



LAC is the Most Powerful Predictor of Outcome



- Predictive power aCL is due to its association with LAC
- Prior thrombosis and presence SLE are predictors of complications

Rethink treatment of aPL+ pregnant patients

Lockshin MD et al. Arthritis Rheum 64:2311-18, 2012

PROMISSE Discovery 2

Historical:

- SLE patients were discouraged from becoming pregnant.

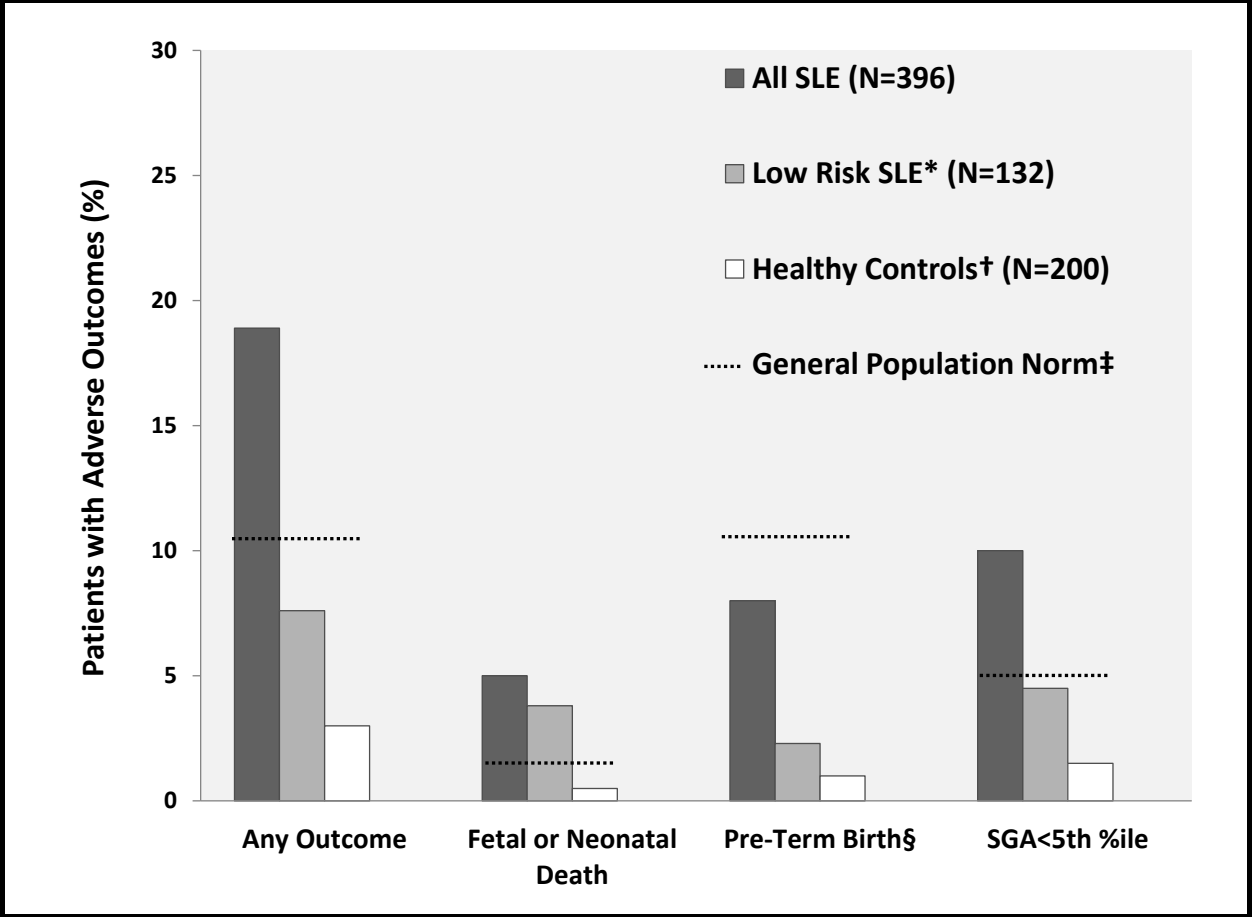
Observation:

- Mild/moderate flares developed in 20.0% (95% CI: 16.1% - 24.3%)
- Severe flares in 6.0% (95% CI: 3.8% - 8.8%)

PROMISSE Discovery 2

Observation:

- Among 396 SLE patients, one or more adverse pregnancy outcomes occurred in 19% of patients, fetal death (4%), neonatal death (1%), preterm delivery (8%), and SGA (10%).

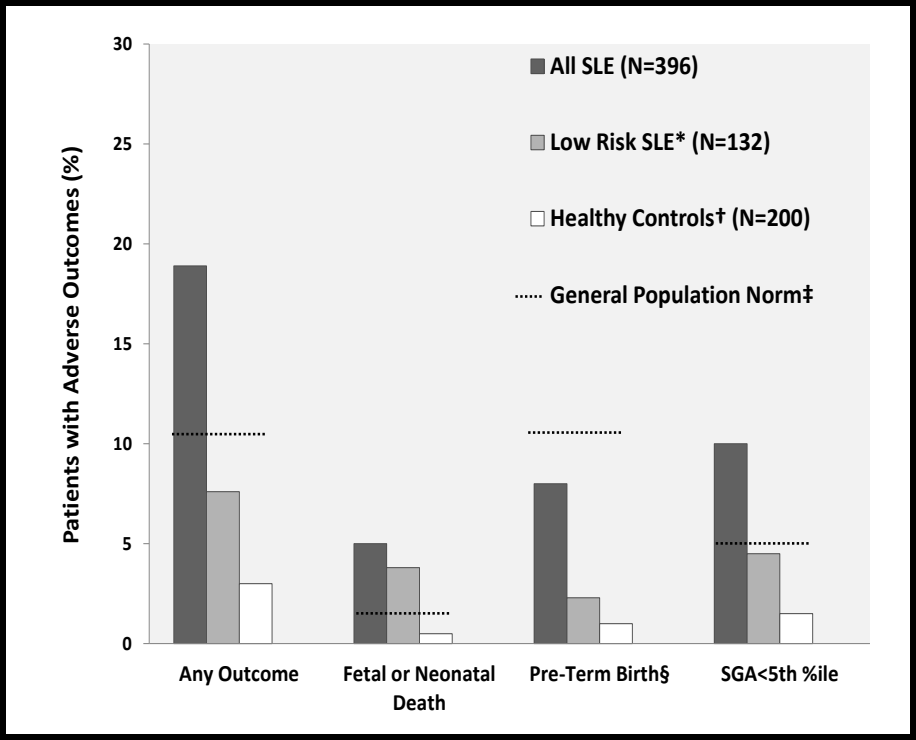


PROMISSE Discovery 2

Baseline predictors of adverse pregnancy outcomes (APO)

- Lupus Anticoagulant (LAC) (OR = 11.55, 95% CI: 4.47-29.86; P <0.0001)
- Antihypertensive use (OR = 6.21, 95% CI: 2.69-14.34; P <0.0001)
- Physician Global Assessment >1 (OR = 3.00, 95% CI: 1.36-6.62; P = 0.007)
- Non-Hispanic White was protective (OR = 0.46, 95% CI: 0.24-0.88; P = 0.02)
- Lower C3 level and flare in 2nd or 3rd trimesters

Among women with no baseline risk factors, APO rate was 7.6% (95% CI: 3.7% - 13.5%)



*** Low risk SLE patients are defined as non-Hispanic White, LAC negative, not moderately or severely active as supported by a PGA <1, and not treated with antihypertensive medications at baseline. They constitute a subset of the "All SLE" group.**

‡ Data were obtained from Roberts JM et al. NEJM 2010; 362:1282-1291.

§ Pre-term birth <36 weeks and indicated by gestational hypertension, preeclampsia, or placental insufficiency

PROMISSE Discovery 2

LIMITATION:

- Excluded patients with high disease activity.

CONCLUSIONS:

- SLE patients with stable, inactive disease can be reassured that severe flares are infrequent, and, in the absence of definable risk factors, pregnancy outcomes are favorable.

PROMISSE Discovery 3

Historical:

- No predictive tests to determine which patients will develop preeclampsia, a serious pregnancy problem.

Observation:

- Circulating anti-angiogenic factors are biomarkers that predict preeclampsia in patients with SLE and/or aPL antibodies.

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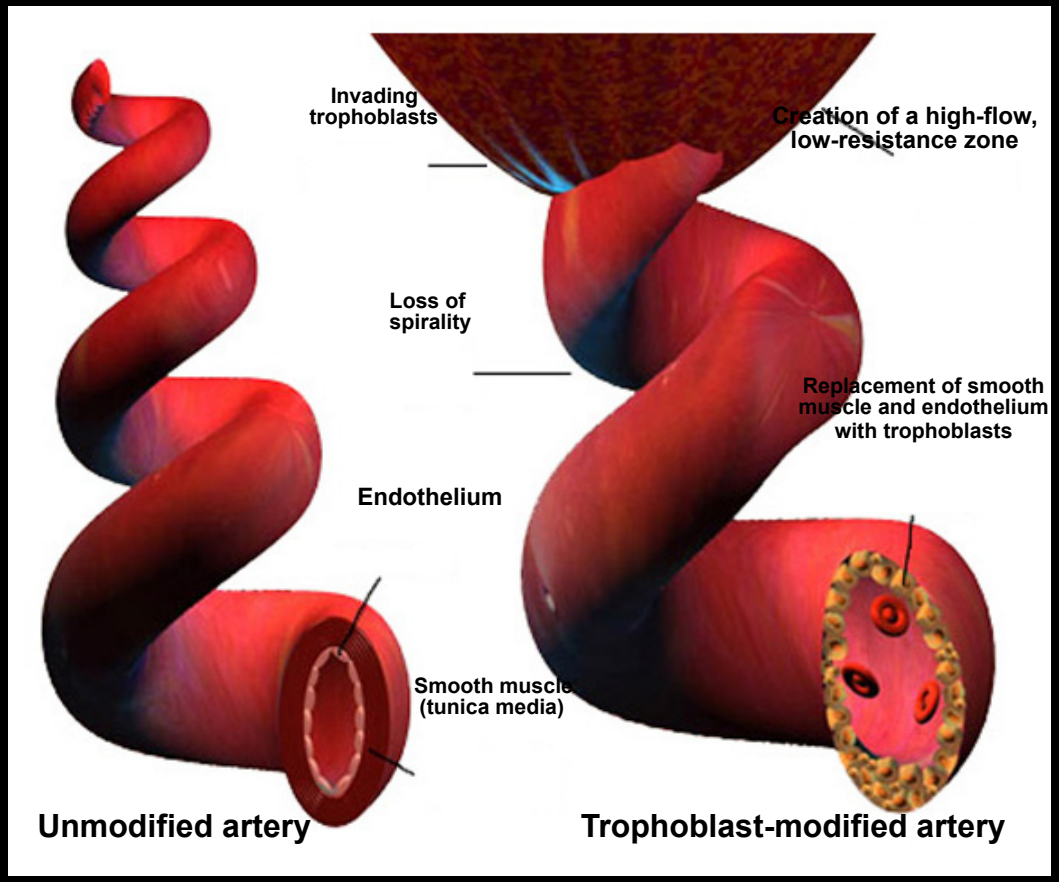
Preeclampsia

A pregnancy-specific disorder defined by the appearance of hypertension and proteinuria, usually after 20 weeks gestation.

- Complicates 4-5% of all pregnancies worldwide
- Claims the lives of > 60,000 mothers each year in developing countries

Preeclampsia

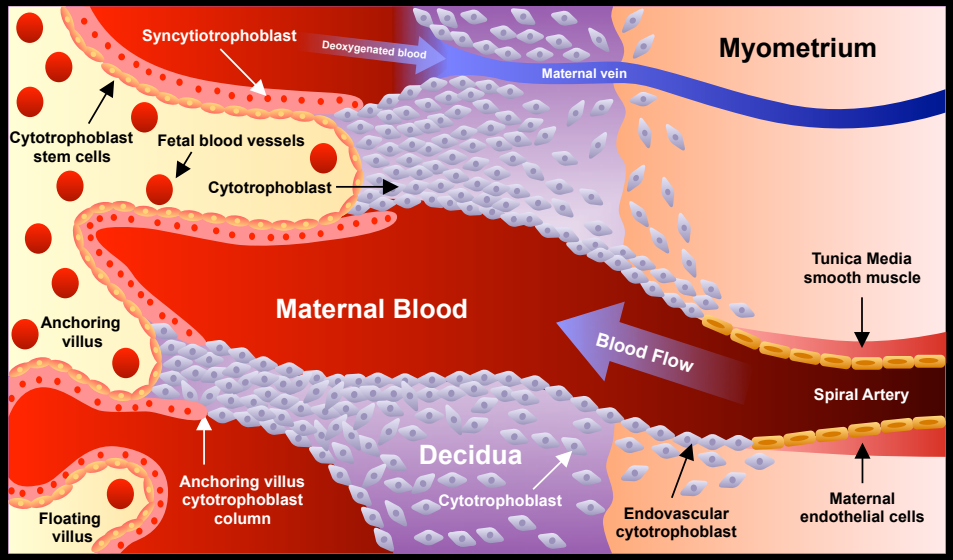
First stage: Abnormal placental development due to a failure of remodeling of uterine spiral arteries into dilated, flaccid vessels in early pregnancy, leading to underperfusion of the placental intervillous space.



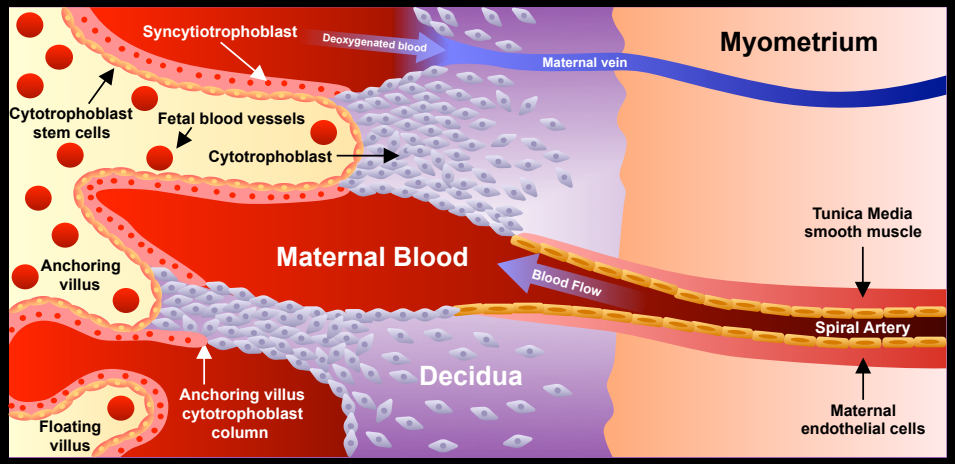
Preeclampsia **Normal**

Abnormal Placentation in Preeclampsia

Normal



Preeclampsia



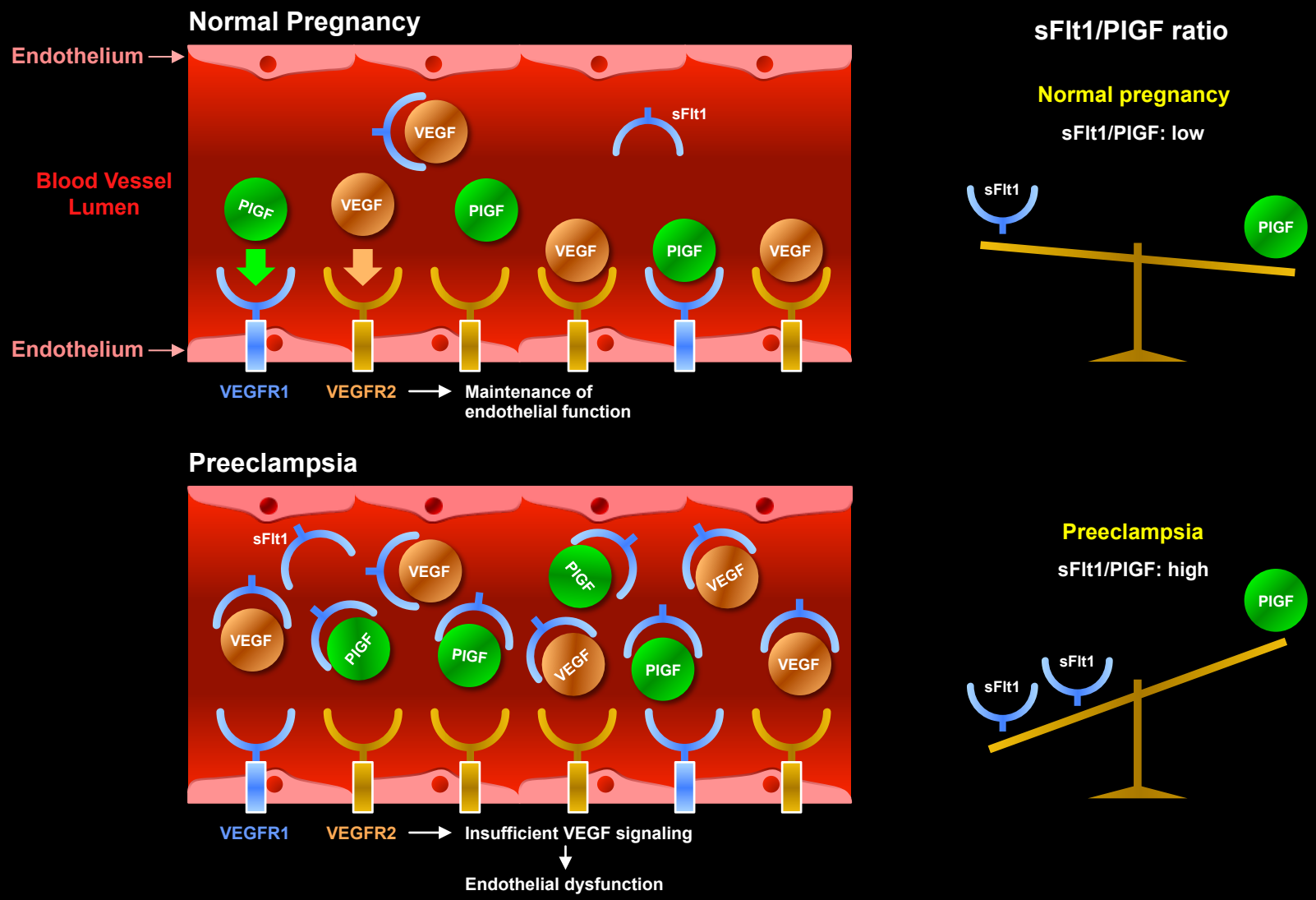
Adapted form Lam C, et al. *Hypertension.* 2005;46:1077-1085.

Preeclampsia

Second stage: Maternal systemic response to placental hypoperfusion, characterized by maternal hypertension, proteinuria, and other end-organ manifestations attributed to widespread **maternal endothelial dysfunction** mediated by placental secretion of **anti-angiogenic factors**.

Picasso, Pregnant Woman (1950) *Hirshhorn Museum and Sculpture Garden, Washington, DC*

Preeclampsia:
A Disorder of Angiogenic Dysregulation



PIGF, placental growth factor; VEGF, vascular endothelial cell growth factor; sFlt1, soluble VEGFR1

Adapted from Hagmann H et al. *Clin Chem.* 2012;58:837

Angiogenic Factors: Experimental Models of Preeclampsia

- Overexpression of sFlt1 in pregnant mice, rats and baboons leads to the classical manifestations of preeclampsia.[1,2]
- Symptoms of preeclampsia (proteinuria and hypertension) and changes in endothelial cell morphology are seen in cancer patients treated with VEGF antagonists.[3]

1. Maynard SE. 2003. J Clin Invest 111: 649
2. Gilbert JS 2007. Hypertension 50:1142
3. Ermina V 2008. N Engl J Med 358:1129

Angiogenic Factors: Prediction and Diagnosis of Preeclampsia

- Cross-sectional case-control studies showed sFlt1 and sFlt1/PlGF ratios to be higher in those destined for preeclampsia[1-4]

  Significant differences occurred 6 weeks or less before clinical manifestations.

- In a small series of SLE patients, sFlt1 measured once between 22-32 weeks was higher in preeclampsia.[5]

1. Levine RJ 2004. N Engl J Med 350:672
2. Levine RJ 2006. N Engl J Med 355:992
3. Noori M 2010. Circulation 122:478
4. Rana S 2012. Circulation 125:911
5. Qazi U 2008. Lupus 35: 631

Hypothesis

Elevated levels of anti-angiogenic factors early in pregnancy predict poor pregnancy outcomes in patients with SLE and/or APL

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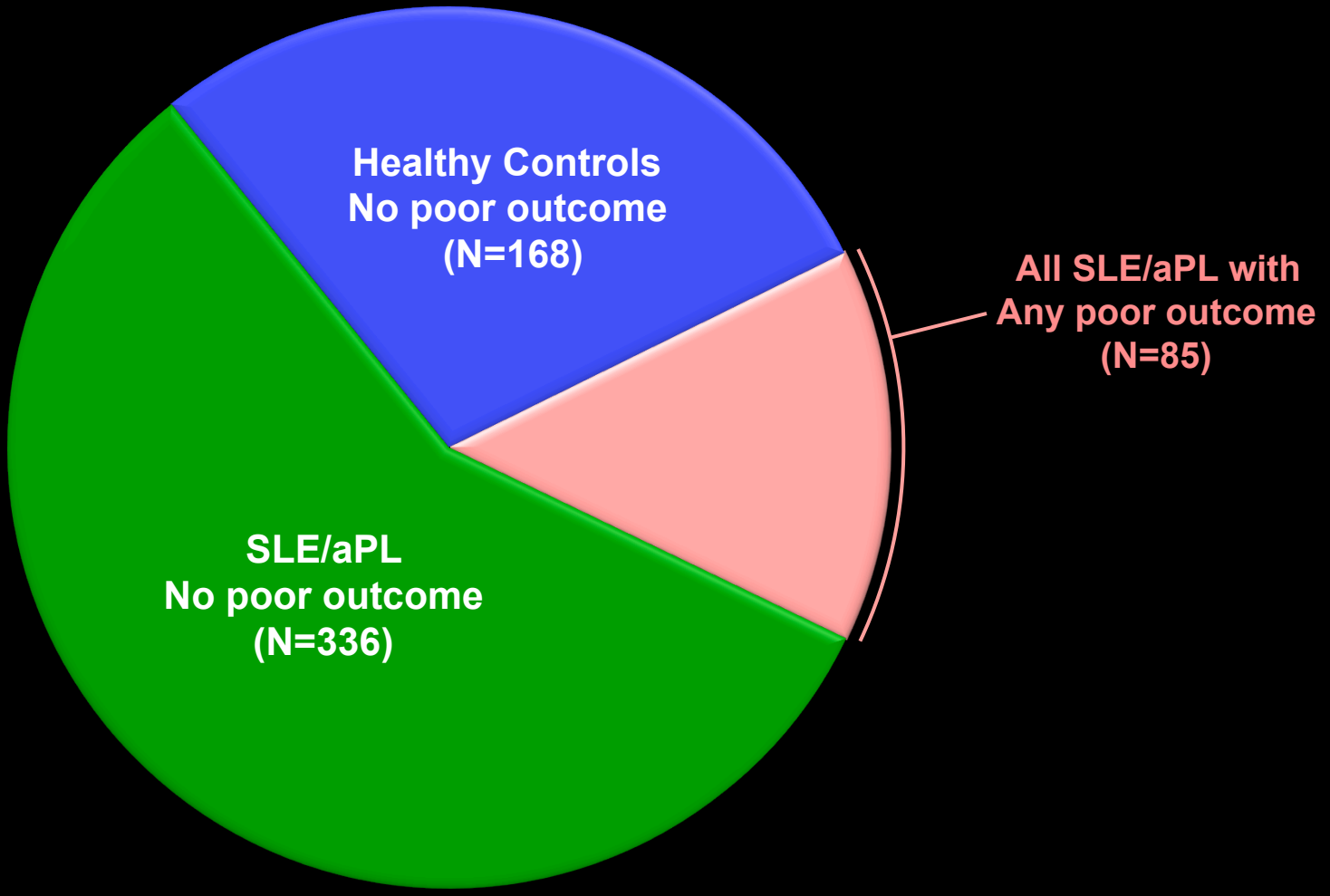
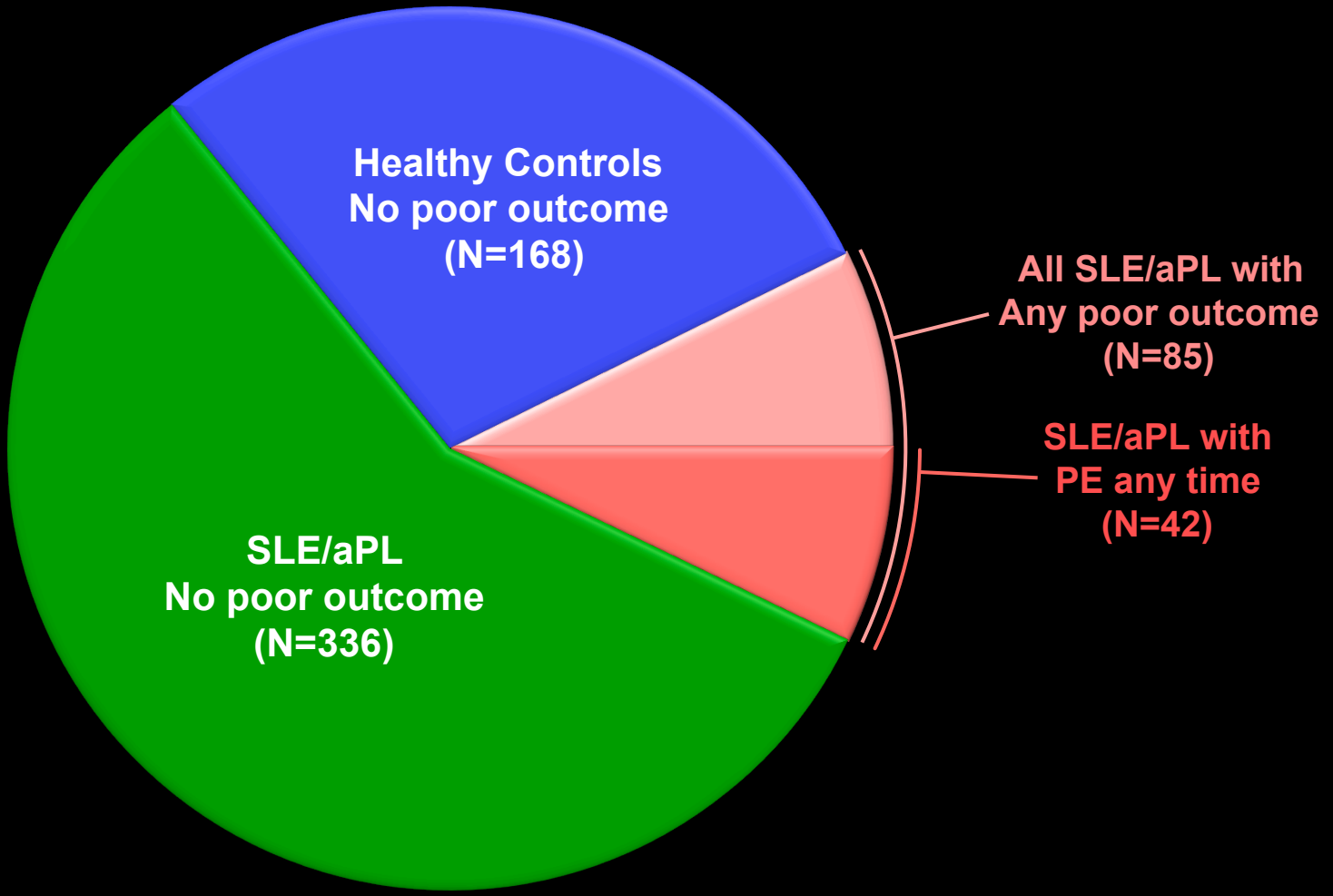
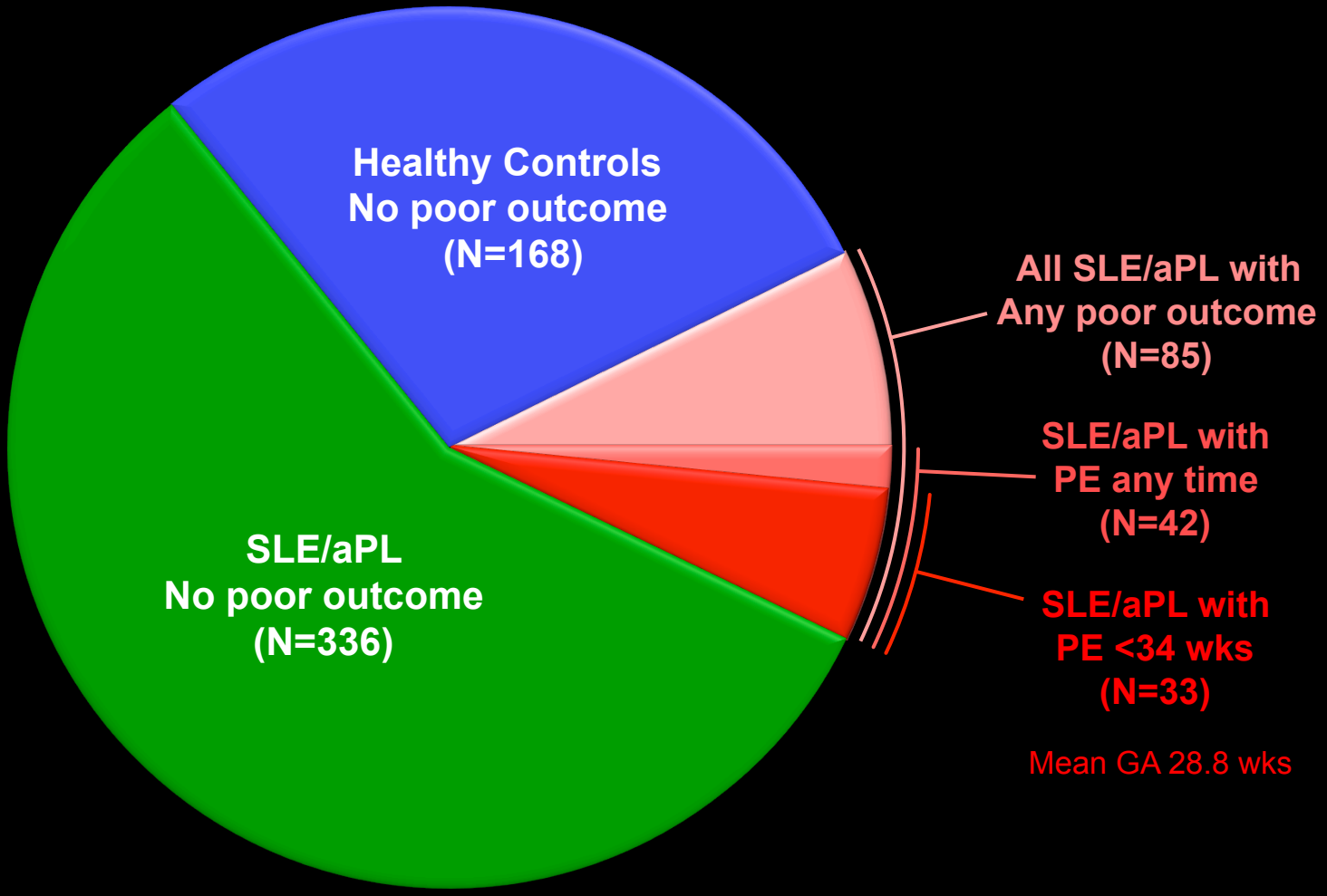
Patient Stratification by Pregnancy Outcome



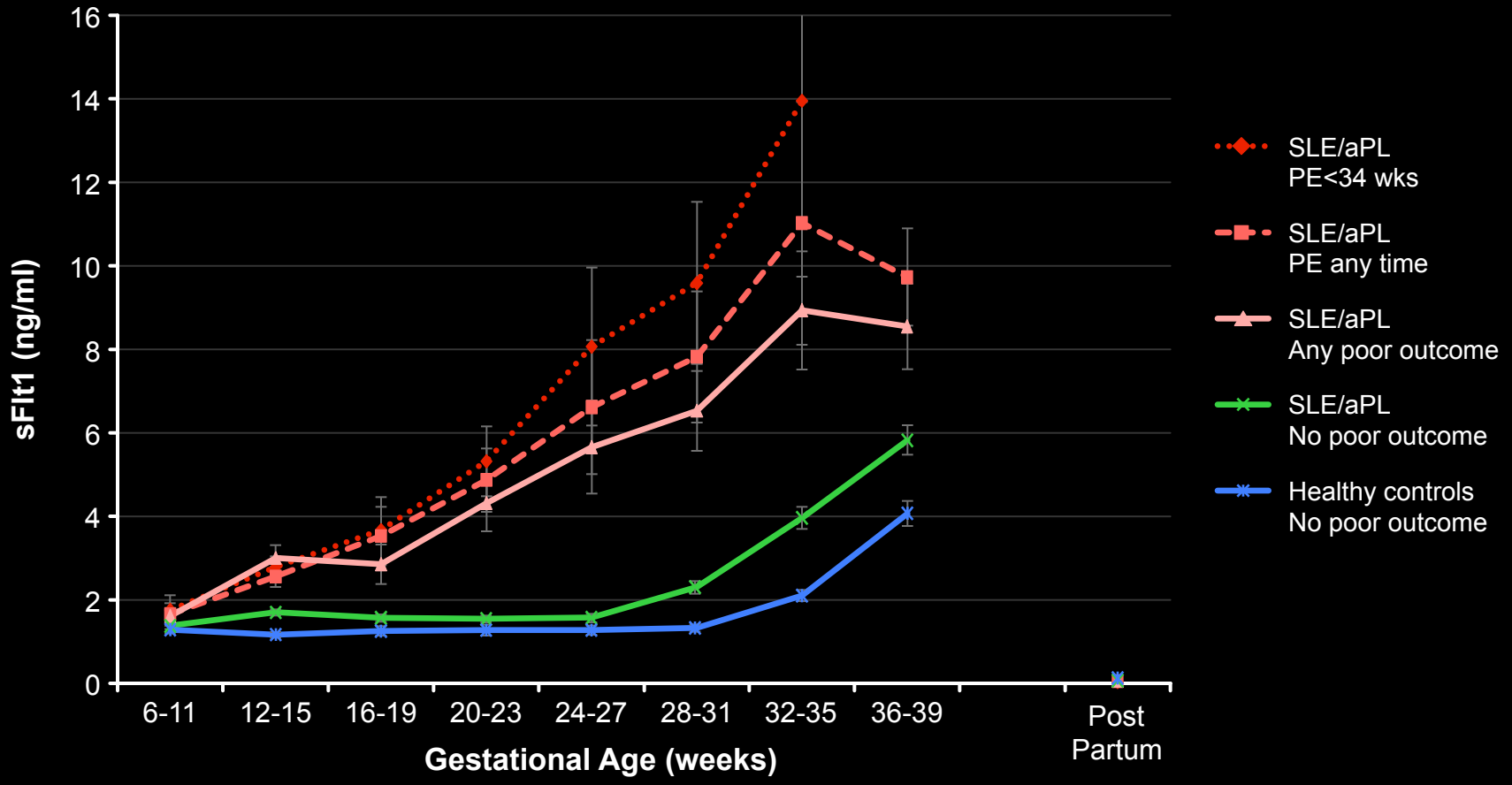
Patient Stratification by Pregnancy Outcome



Patient Stratification by Pregnancy Outcome

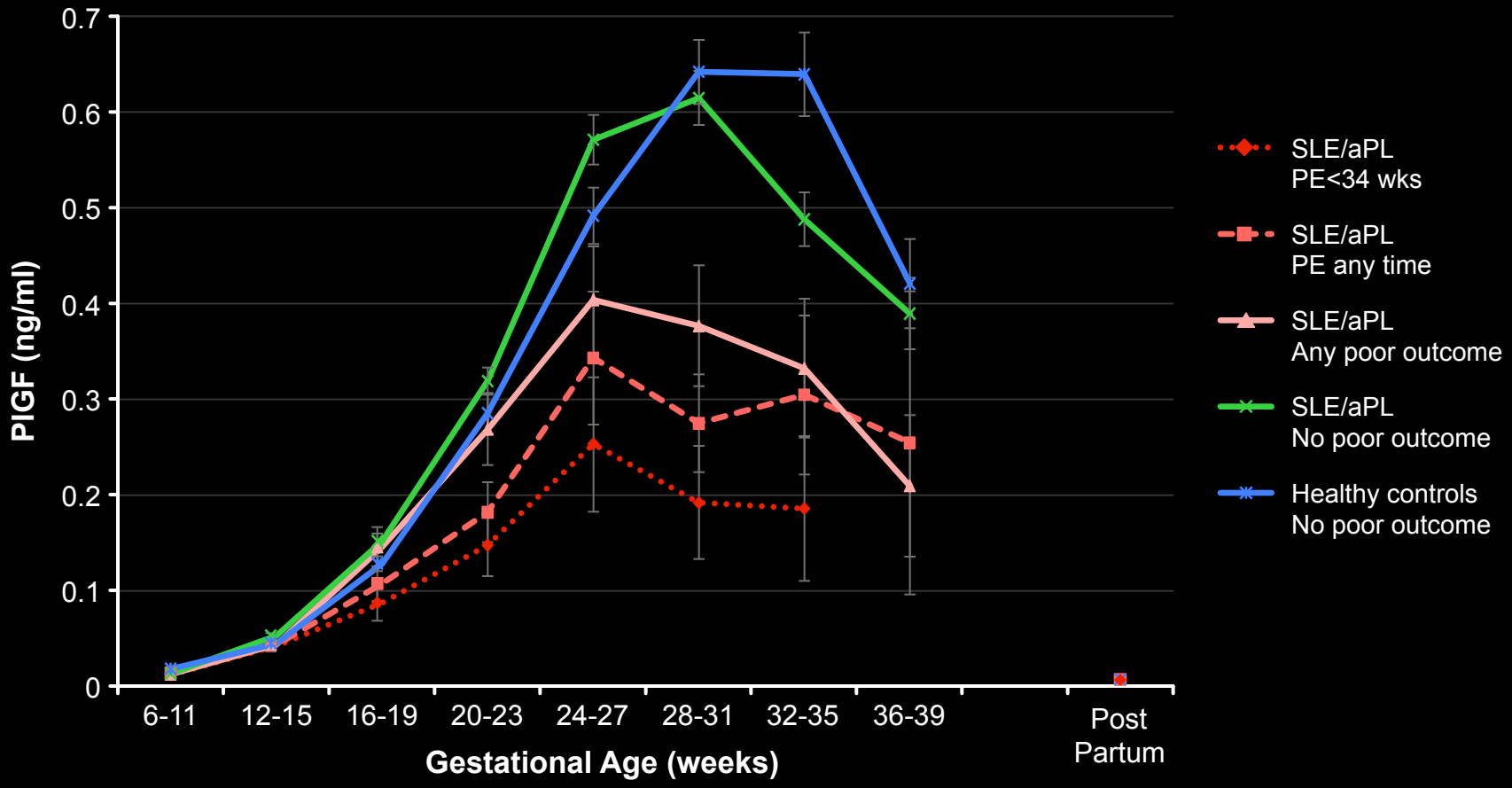


Elevated sFlt1 Precedes Poor Outcomes



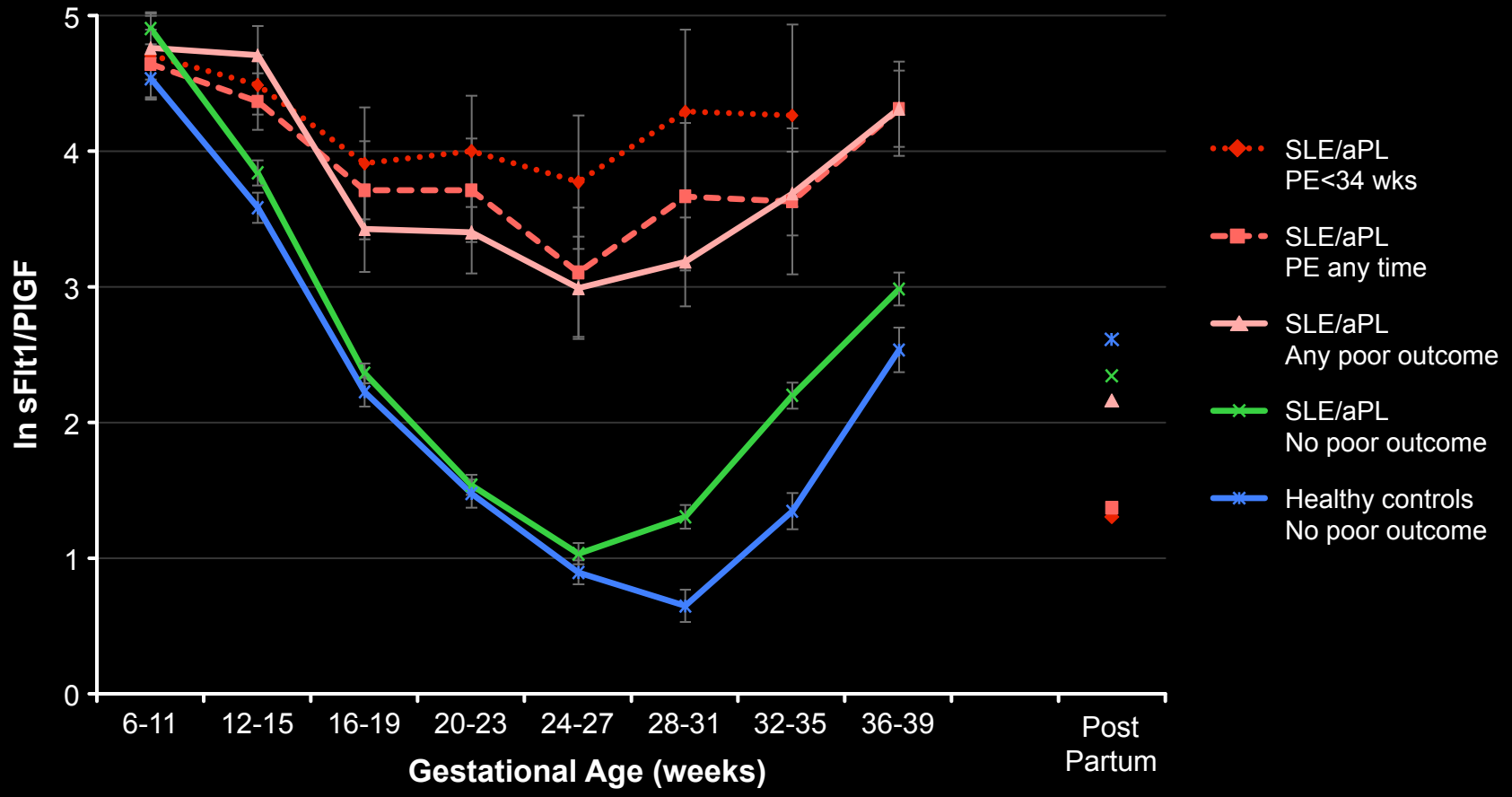
- sFlt1 was significantly higher from 12 - 31 weeks in SLE/aPL patients with PE<34wks, PE anytime, and any poor outcomes compared to SLE/aPL patients with no poor outcome
- Rate of increase in sFlt1 from 12 - 31 weeks was higher in SLE/aPL patients with PE<34wks, PE anytime, and any poor outcome compared to SLE/aPL patients with no poor outcome ($p < 0.0001$ all comparisons)

Lower PIGF is Associated with Poor Outcomes



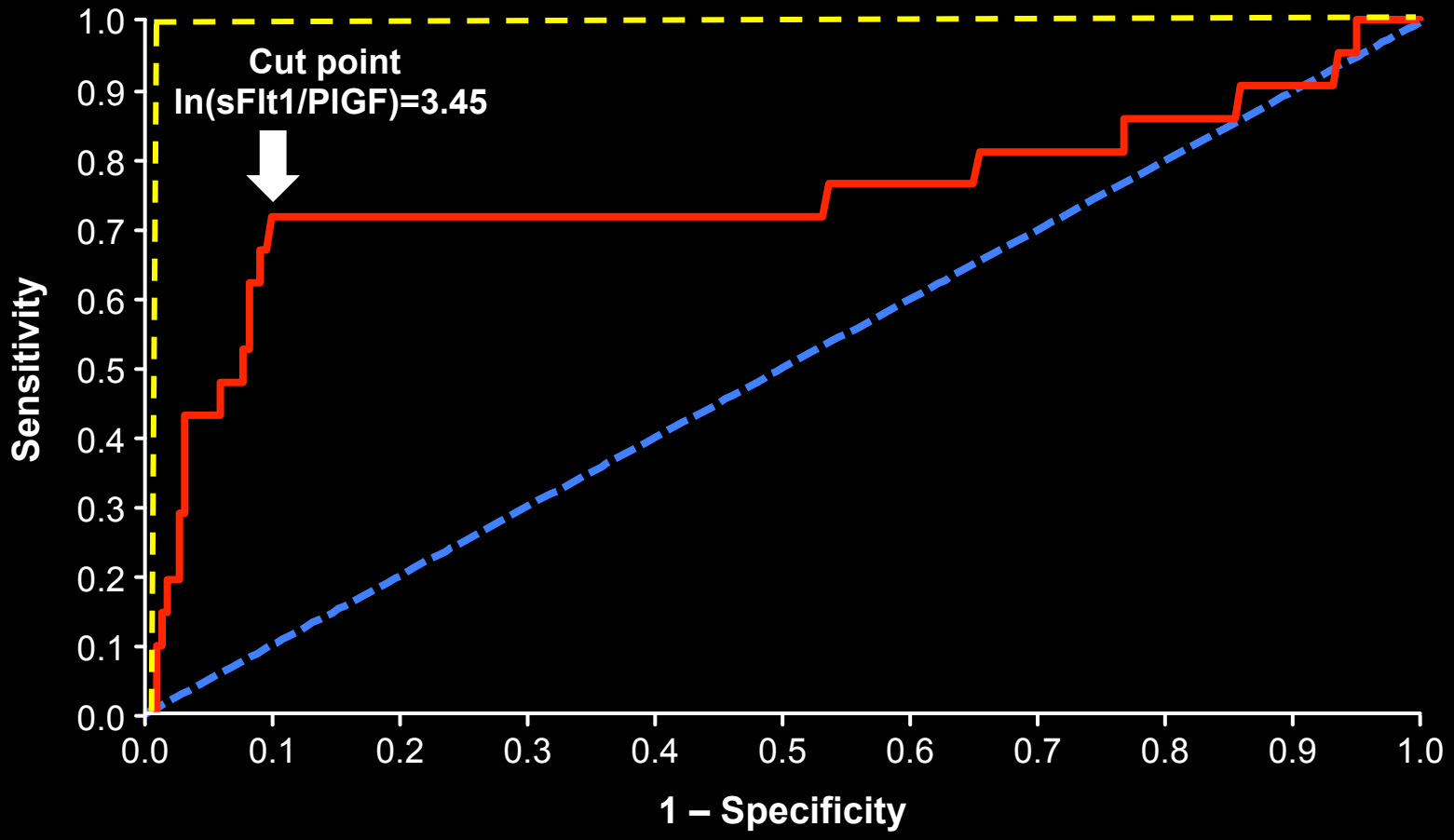
- PIGF was significantly lower in most comparisons from 16 - 31 weeks in SLE/aPL patients with PE<34 wks, PE anytime, and any poor outcomes compared to SLE/aPL patients with no poor outcome
- Rate of increase in PIGF from 12 - 31 weeks was lower in SLE/aPL patients with PE<34 wks, PE anytime, and any poor outcome compared to SLE/aPL patients with no poor outcomes ($p < 0.0001$ all comparisons)

Elevated sFlt1/PlGF is Associated with Poor Outcome



- Ln (sFlt1/PlGF) was significantly higher from 12 - 35 weeks in SLE/aPL patients with PE<34 wks, PE anytime, and poor outcomes compared to SLE/aPL patients with no poor outcome
- Rate of decrease in ln(sFlt1/PlGF) from 12 - 31 weeks was less in SLE/aPL patients with PE<34 wks, PE anytime, and no poor outcomes compared to SLE/aPL patients with no poor outcomes ($p < 0.0001$ all comparisons)

ROC Curve for PE<34 weeks using ln(sFlt1/PlGF) at 16-19 weeks' gestation



Area Under the Curve = 0.74

Performance of ln(sFlt1/PlGF) > 3.45 at 16-19 weeks as a Screening Test for PE<34 weeks

Sensitivity (true pos)	71%
Specificity (true neg)	90%
Positive Predictive Value	41%
Negative Predictive Value	97%
Relative Risk	13.8 (95% CI 5.7-33.2)

All subjects with aPL/SLE
Comparison group = aPL/SLE without PE<34 weeks

Conclusion

- In SLE and/or aPL-positive patients, alterations in the balance of angiogenic factors early in pregnancy are strongly associated with subsequent preeclampsia and other poor outcomes.
- That angiogenic imbalance is associated with a spectrum of poor pregnancy outcomes (e.g., growth restriction, preterm delivery, etc.) suggests common pathogenesis.
- Low sFlt1/PlGF ratios, as well as low sFlt1 or high PlGF levels, can reassure physicians and patients that preterm preeclampsia is unlikely.
- Ratios of sFlt1/PlGF may be used as early 16-19 weeks' gestation for risk stratification in future prevention trials.

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PROMISSE Discovery 4

- Mutations in complement pathway genes that lead to uncontrolled complement activation are associated with preeclampsia in pregnant patients with SLE and/or aPL antibodies.

Salmon et al. PLoS Med, 2011

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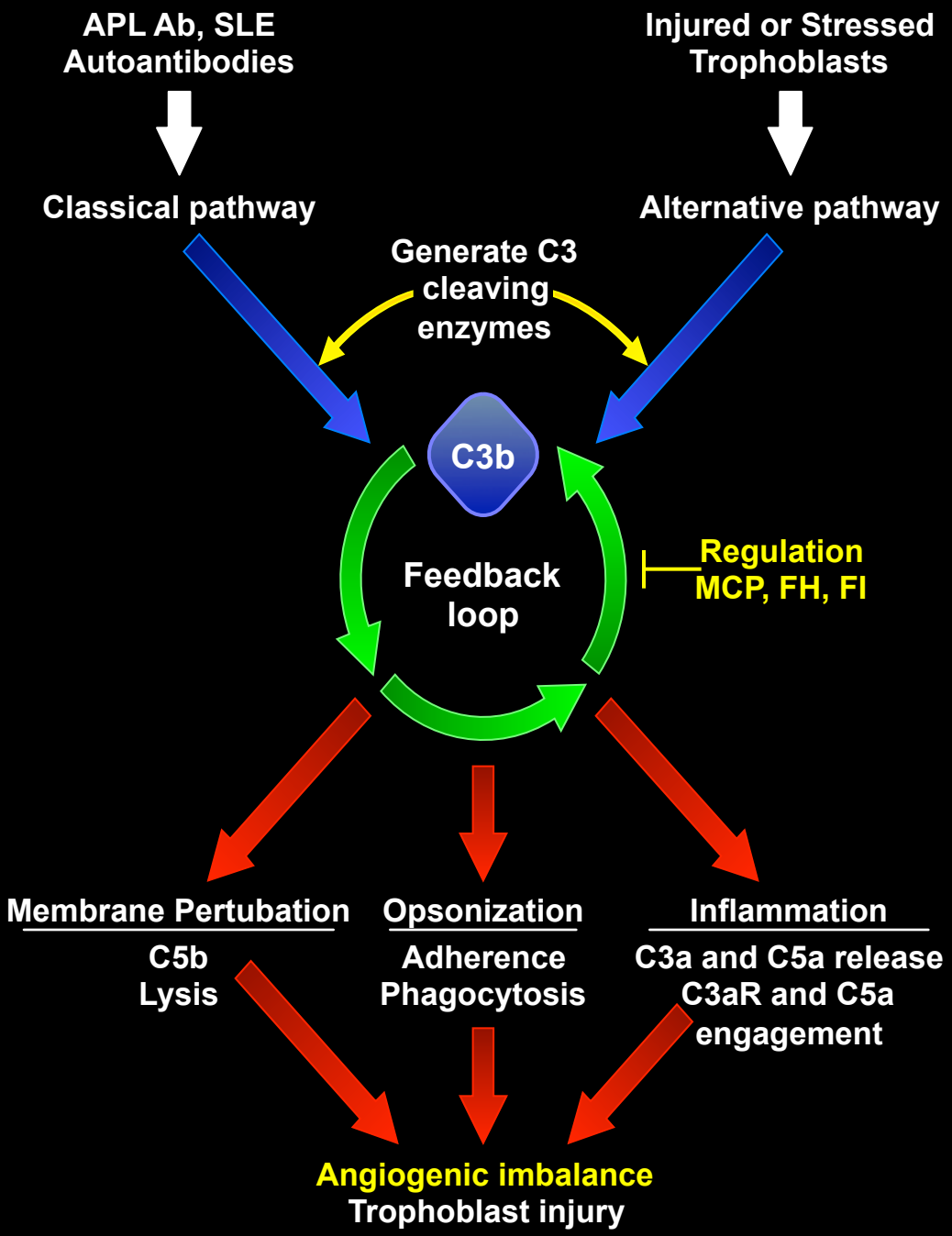
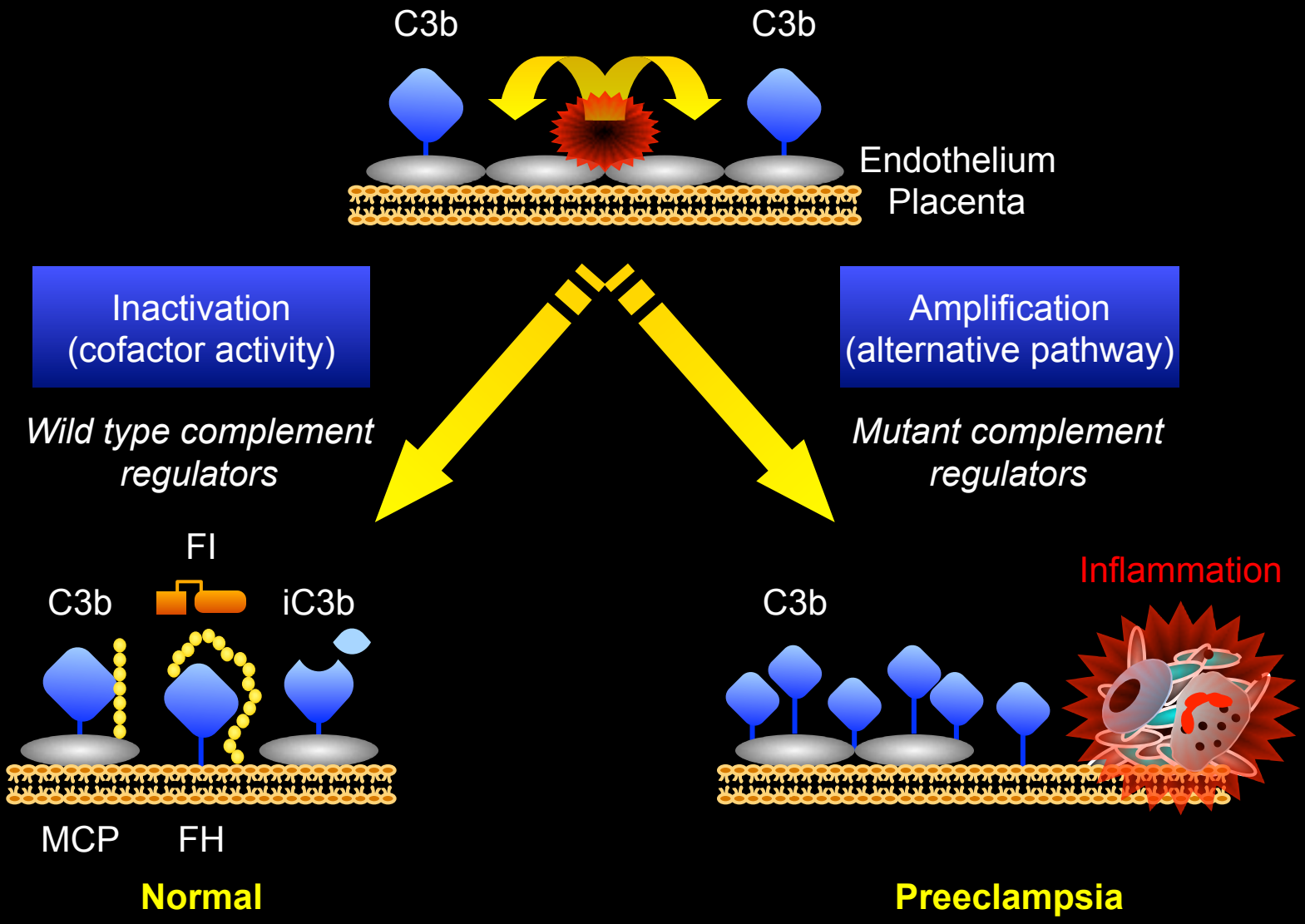


Salmon et al. PLoS Med, 2011



Adapted from Richards A et al. *Adv Immunol.* 2007;96:141-77.

The PROMISSE Study

Predictors of P**R**egnancy **O**utcome:
Bio**M**arkers **I**n Antiphospholipid **S**yndrome and **S**ystemic Lupus **E**rythematosus

To be continued……

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Acknowledgments

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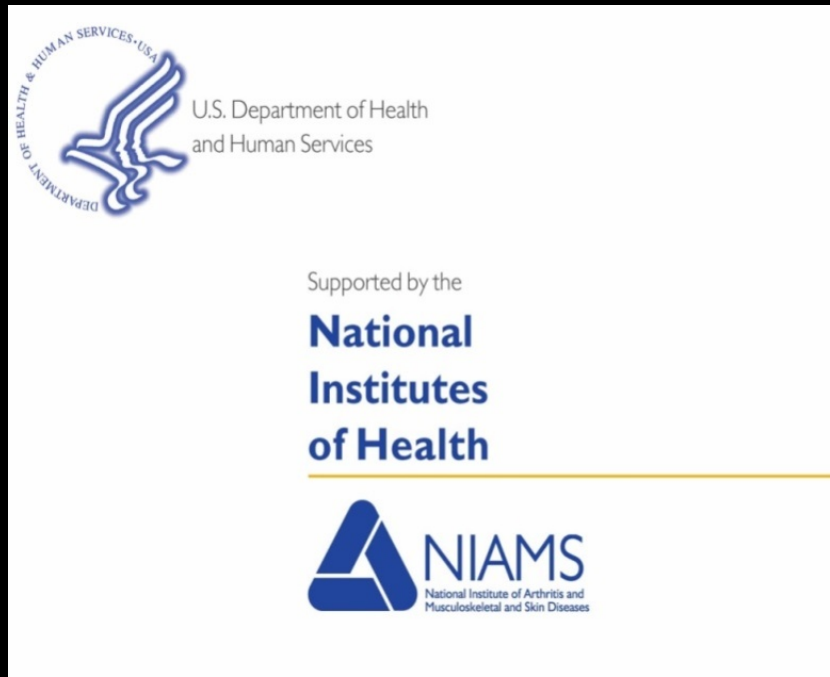







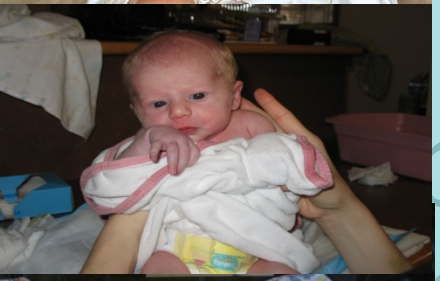
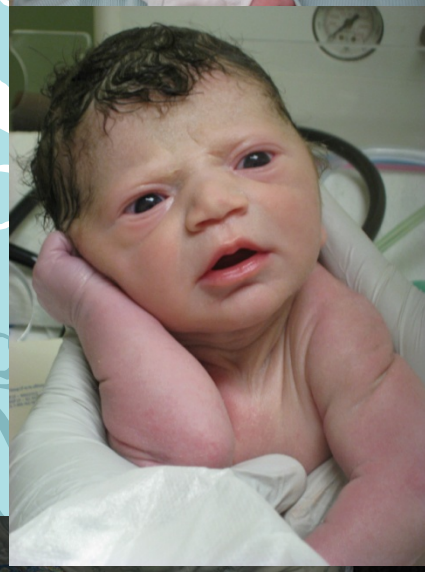

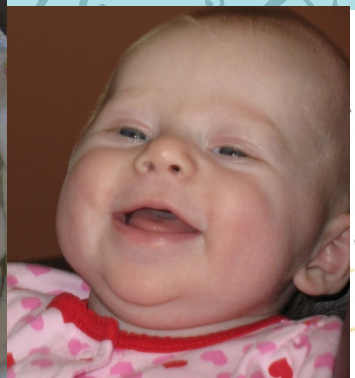
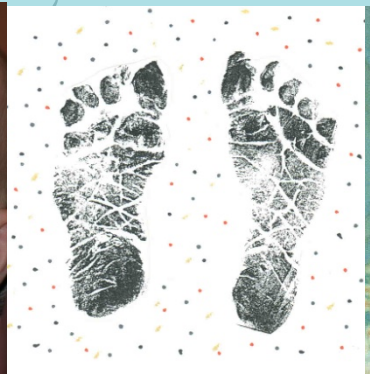
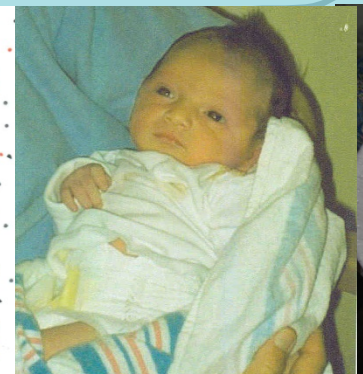


Thank you

PROMISSE

Babies