Clinical implementation of cell-free DNA testing for trisomy detection

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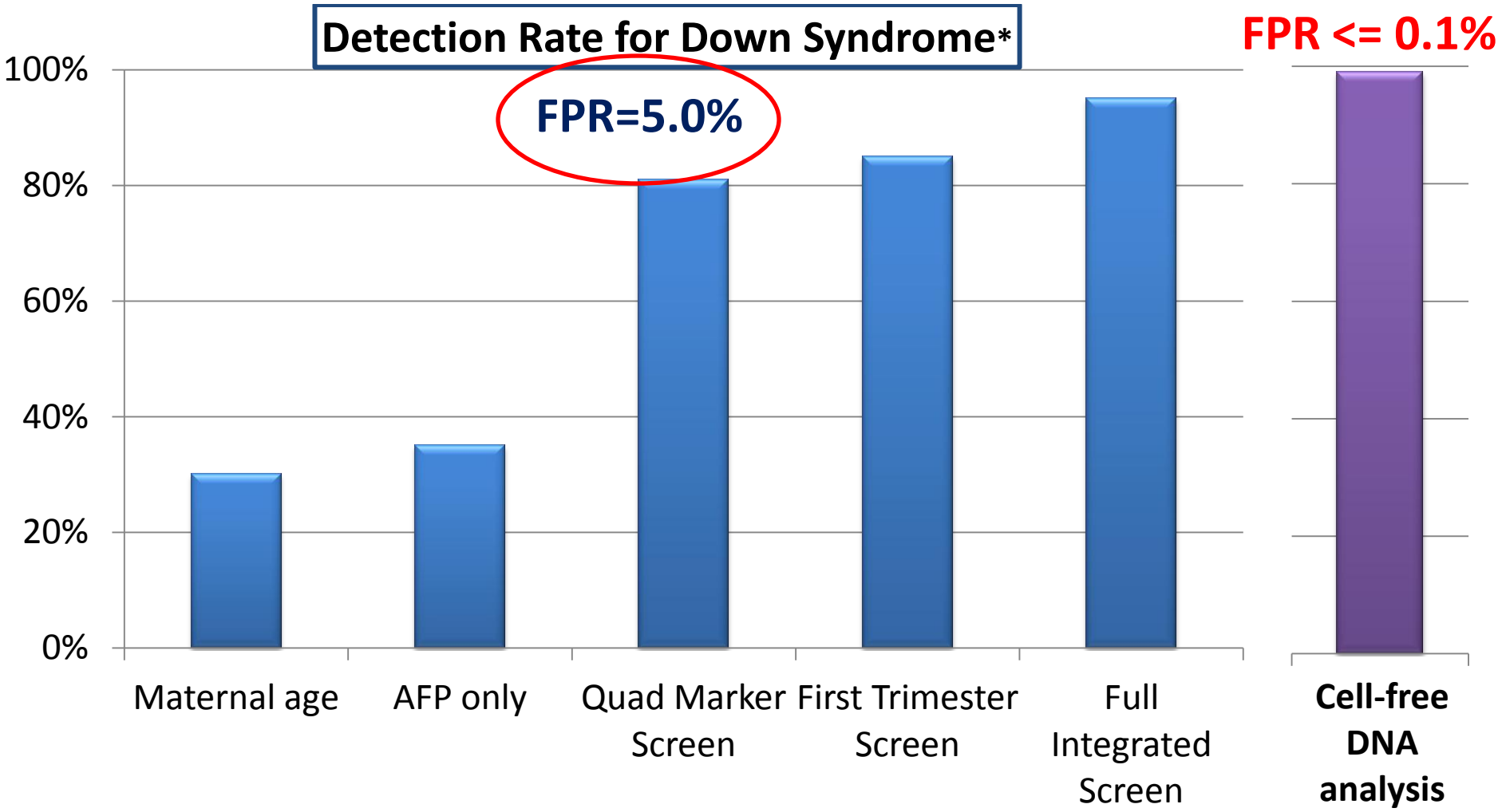
CENTRO ANALISI MONZA

«DIAGNOSI PRENATALE NON INVASIVA:
scelte cliniche in equilibrio tra nuovi orizzonti»
September 2013
Monza, Italy

Evolution of Trisomy Assessment

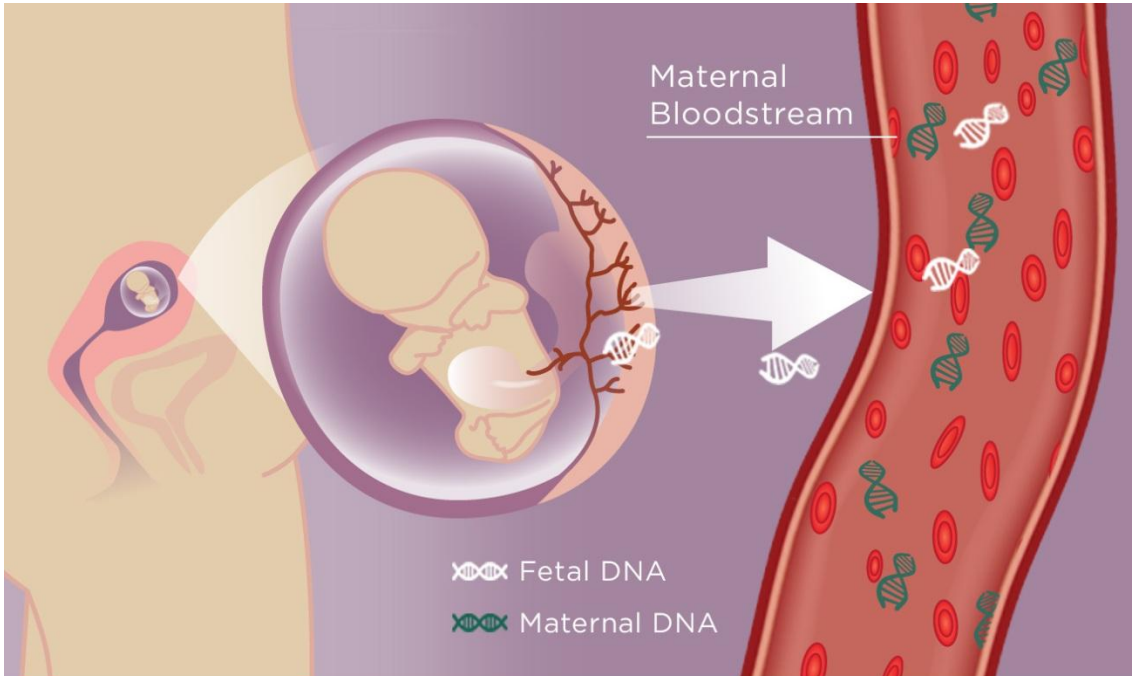


1970's → 1980's → 1990's → 2000 →→→

*ACOG practice bulletin no. 77, Obstet Gynecol 2007;109:217-27.

Cell-free DNA in Maternal Blood

- As cells turnover, chromosomes fragment, releasing DNA into the blood
- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mom and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA



NIPT Performance – cfDNA Methods

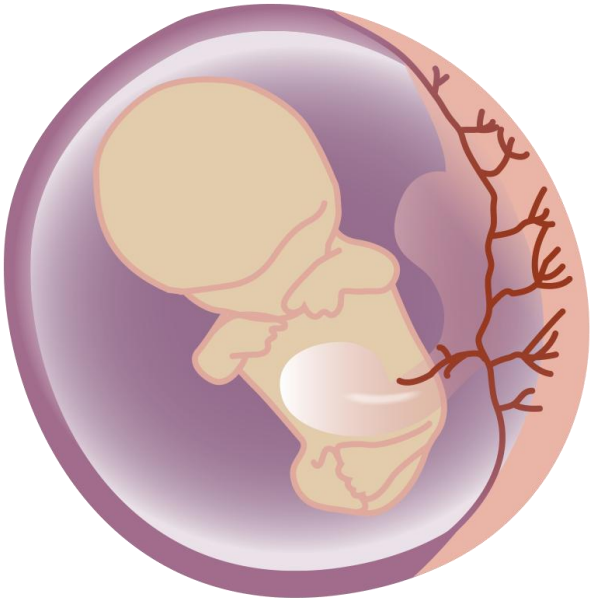
	Detection rate	FPR
Trisomy 21	590 / 594 (>99%)	0.1%
Trisomy 18	222 / 230 (97%)	0.1%
Trisomy 13	30 / 38 (79%)	0.1%

Chiu et al, 2011;
Chen et al, 2011;
Ehrich et al, 2011;
Palomaki et al, 2011;
Bianchi et al, 2012;
Sparks et al, 2012;
Ashoor et al, 2012;
Norton et al, 2012

cfDNA does not always correlate with fetal genotype
(placental mosaicism, vanishing twin, maternal mosaicism)

Trisomy 18 and 13 – discordance

Explaining "false positive" and "false negative" NIPT results



- cfDNA originates from placenta
  - Likely to be from trophoblast
  - May be similar to "Direct prep" of chorionic villi
- Chromosomal makeup of placenta and fetus can be different
- Occurs more frequently with chromosomes 13 and 18, as compared to chromosome 21
  - T13 and T18 (Kalousek et al)*
    - Only 30% of trophoblast showed trisomy when other fetal tissue 100% for trisomy 13 and 18 (viable)
  - Recent case report of discordance between NIPT and fetus for trisomy 13
    - NIPT: "positive" for T13
    - CVS: mosaic 47,XY,+13[10]/46,XY[12]
    - Amnio normal, fetus normal
    - Placental biopsies = 2/4 mosaic trisomy 13

* Kalousek DK et al., Am J Hum Genet. 1989 Mar;44(3):338-43.

**Hall AL, Drendel HM, Verbrugge JL, Reese AM, Schumacher KL, Griffith CB, Weaver DD, Abernathy MP, Litton CG, Vance GH, Genetics in Medicine (2013)

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Ashoor et al, 2012;
Norton et al, 2012

It is still a screening test……

NIPT for T21 – Not Diagnostic

- NIPT results need to be taken in the context of disease prevalence

NIPT example:

Test accuracy:

99% detection

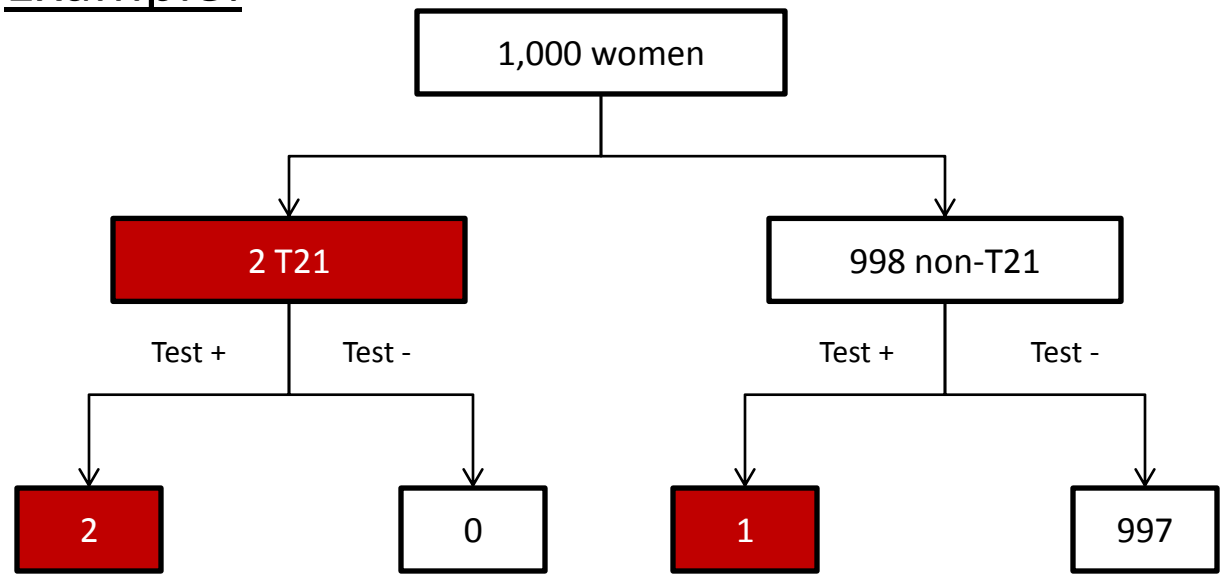
0.1% false positive

T21 prevalence:

1 in 500*

*mid-trimester risk of 32 yo

Example:



Positive test result is correct only 2/3 of the time

In this example: Postive Predictive Value = 66% Negative Predictive Value > 99%

NIPT for T21 – Not Diagnostic

- NIPT results need to be taken in the context of disease prevalence

Harmony Test accuracy:

>99% detection

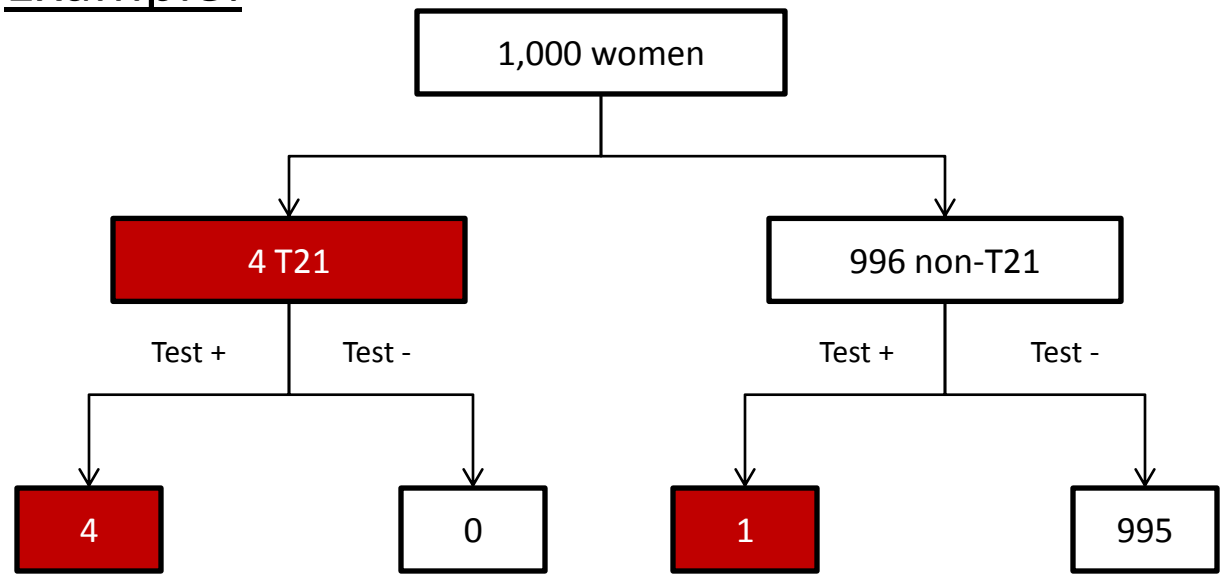
0.1% false positive

T21 prevalence:

1 in 250*

>1:100 = "screen positive"

Example:



*mid-trimester risk of 35yo

Positive test result is correct 4/5 of the time

1st Trim Screen PPV = 6%

PPV = 80% NPV >99%

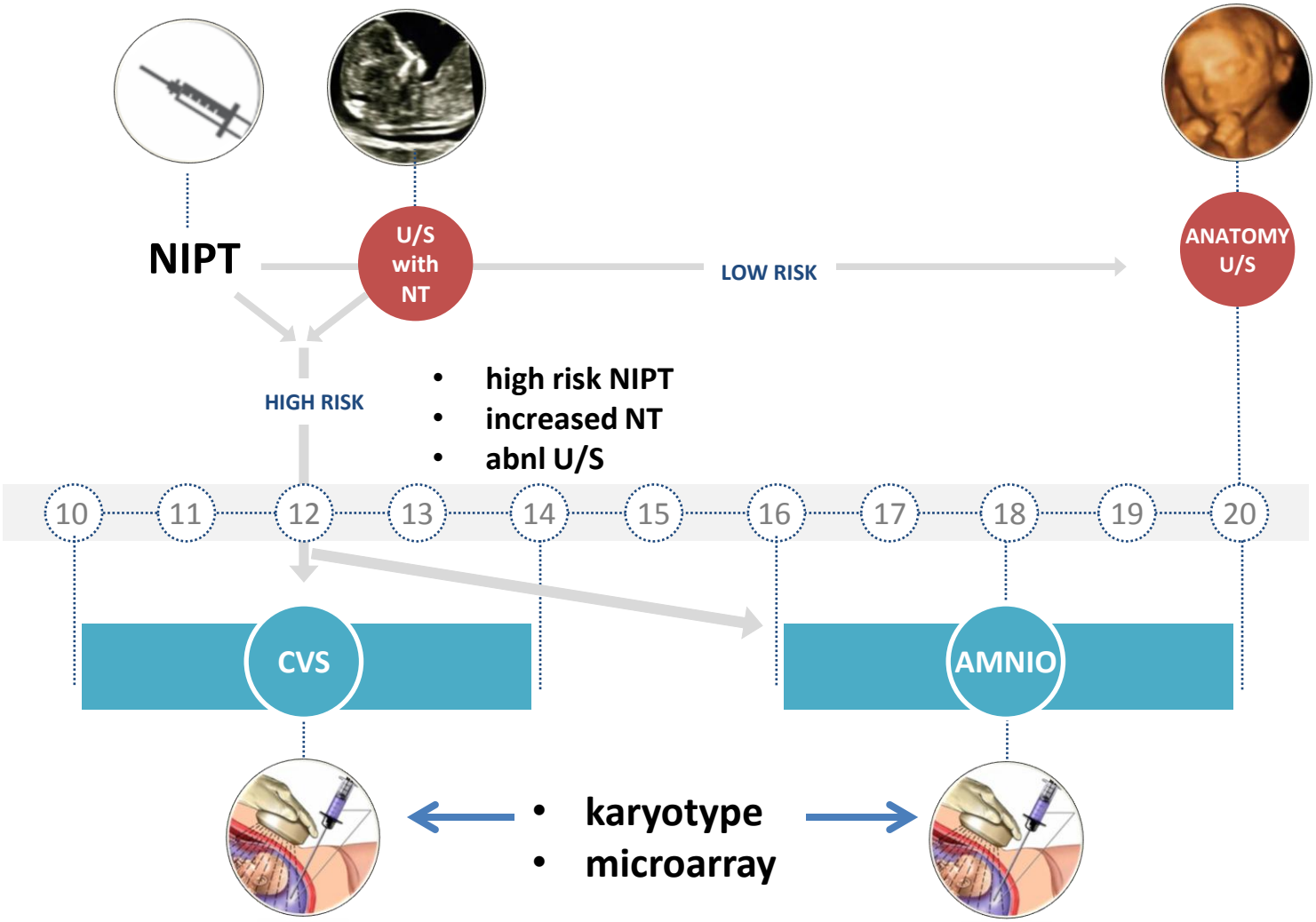
Where does non-invasive prenatal testing (NIPT) fit within current clinical practice?

Clinical implementation of NIPT

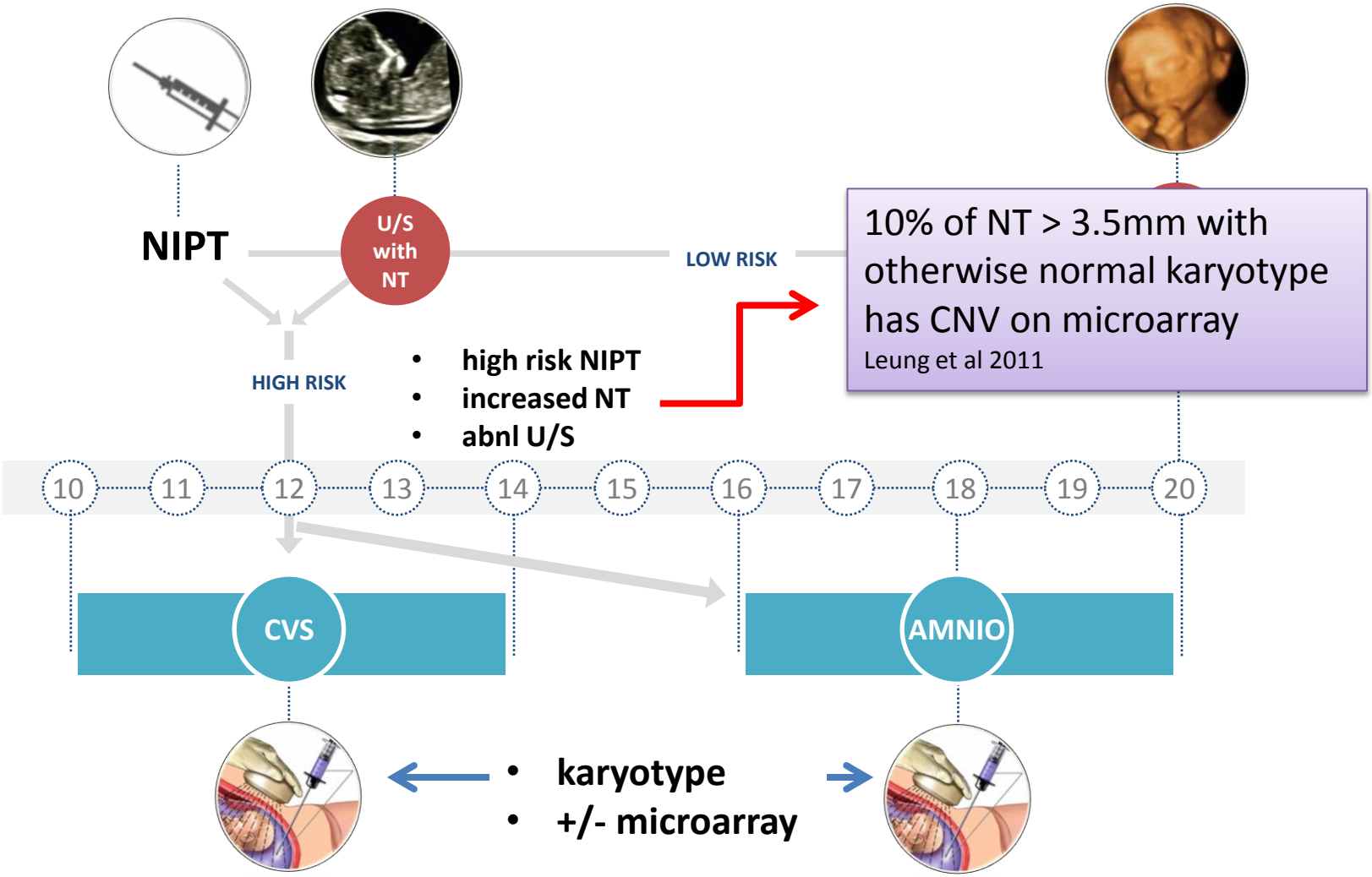
- Alternative to 1st trimester serum screening
  - NT first - then stratify risk
  - NT + NIPT – then stratify risk
- Alternative to 2nd trimester quad screen
  - Increased Detection rate for patients that book late to care and miss 1st trimester window
- "Contingent" screening model
  - Retain serum screening + NT, set cut-off for NIPT
  - May be appropriate for public funded programs or where resources are limited
  - Modeled in papers by:
    - Nicolaides et al 2013: 1 in 3,000 cutoff (FTS model)
    - Wald et al 2013: 1 in 1,600 (Integrated screening model)

Replacement for serum screening

Screen alternative: NIPT + 1st trimester ultrasound



Screen alternative: NIPT + 1st trimester ultrasound



AJOG American Journal of Obstetrics & Gynecology

REPORTS OF MAJOR IMPACT www.AJOG.org

Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population

Kypros H. Nicolaides, MD; Argyro Syngelaki, RM; Ghalia Ashoor, MD; Cahit Birdir, MD; Gisele Touzet, MD

★ EDITORS' CHOICE ★

Am J Obstet Gynecol. 2012 Nov;207(5):374.e1-6 (Epub 2012 Sep 19)

Study objective

- **Compare the clinical accuracy of:**

NIPT (cfDNA)
- **directed sequencing assay**
- **Risk score for trisomy 21 and 18**

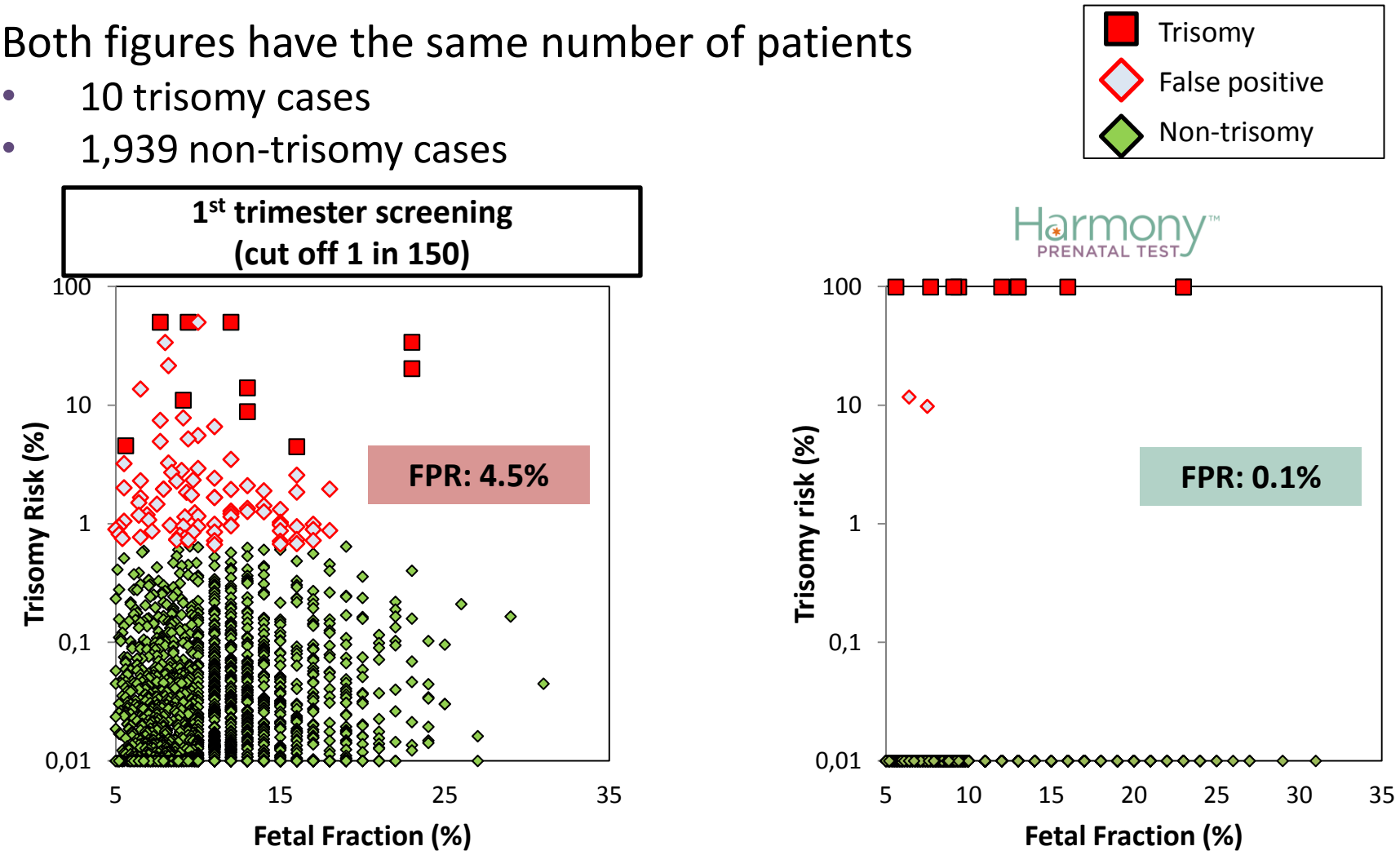
versus

1st trimester combined screening
- **serum markers**
- **nuchal translucency (NT) measurement**

Nicolaides et al, Am J Obstet Gynecol. 2012 Nov;207(5):374.e1-6 (Epub 2012 Sep 19)

Results – Risk Score Comparison

- Both figures have the same number of patients
  - 10 trisomy cases
  - 1,939 non-trisomy cases

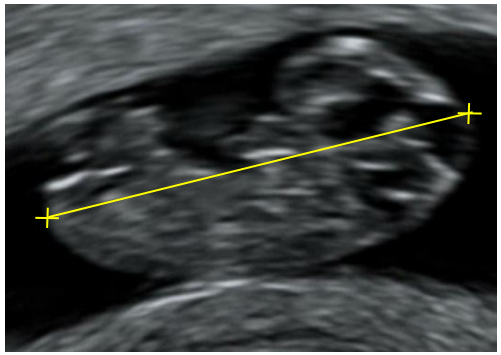


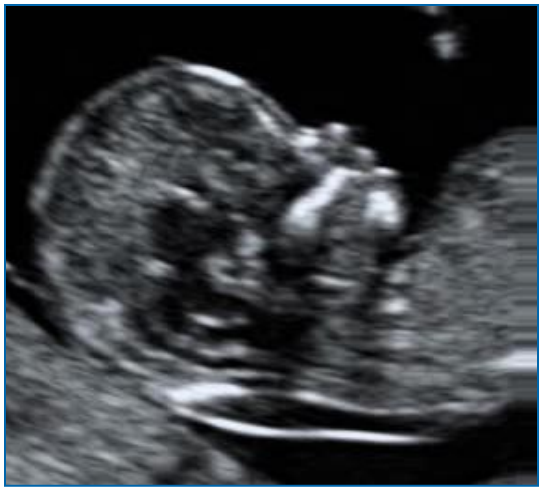
Nicolaides KH et al., Am J Obstet Gynecol. 2012 Nov;207(5):374.e1-6.

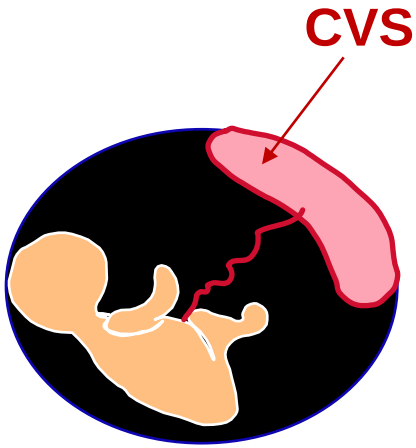
Implementation of maternal blood cell free DNA testing in early screening for aneuploidies.

Gil, Quezada, Bregant, Ferraro, and Nicolaides. Ultra Obstet Gyn, 2013

The Fetal Medicine Centre







10 weeks:

- Scan to measure the fetus
- Blood for cfDNA test- shipped to California
- Blood for combined test

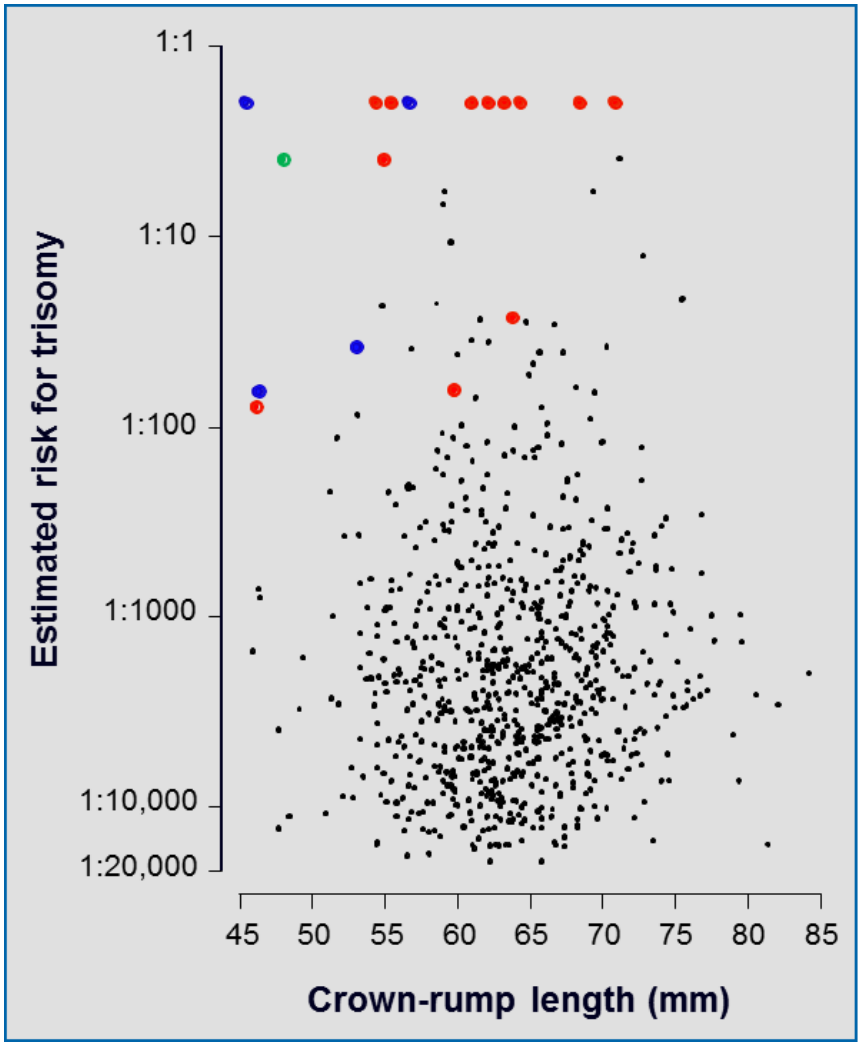
12 weeks:

- Detailed ultrasound scan
- Nuchal Translucency
- Discuss results
- Decide if CVS is necessary

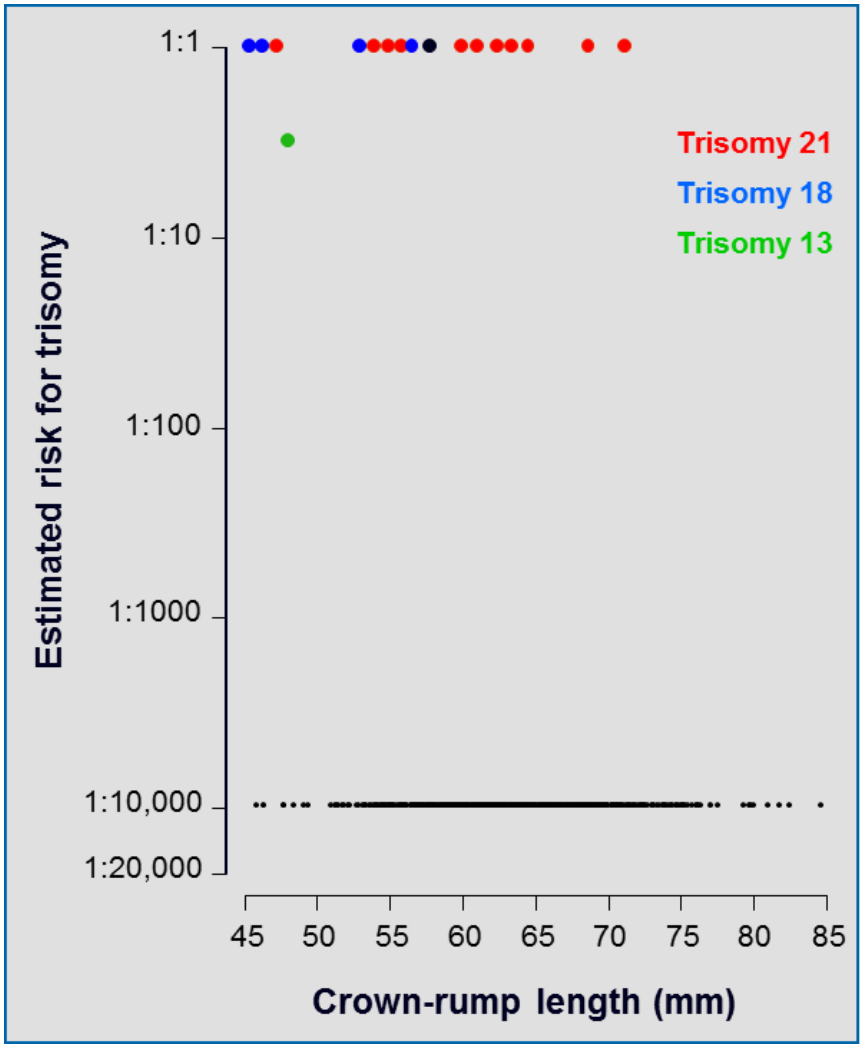
- high risk cfDNA test
- Fetal defects
- NT ≥3.5 mm

Risk comparison: CST v. NIPT

Gil, Quezada, Bregant, Ferraro, and Nicolaides. Ultra Obstet Gyn, 2013



1st Trimester Combined Screening Test



HARMONY Prenatal Test

PRENATAL DIAGNOSIS

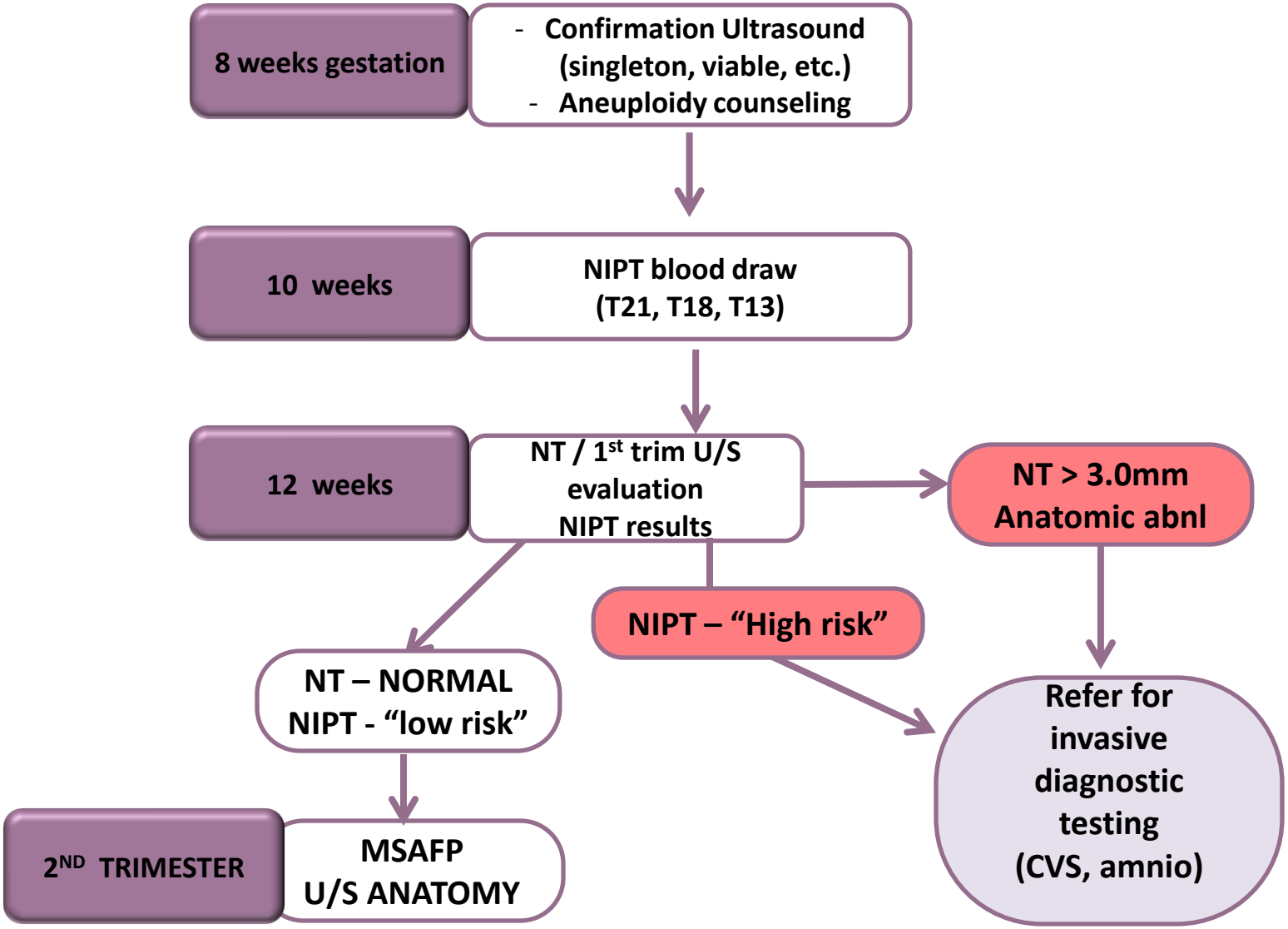
Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population

Fairbrother G , Johnson S, Musci TJ and Song S

Article published online: 15 MAR 2013

Clinical Implementation (Atlanta)

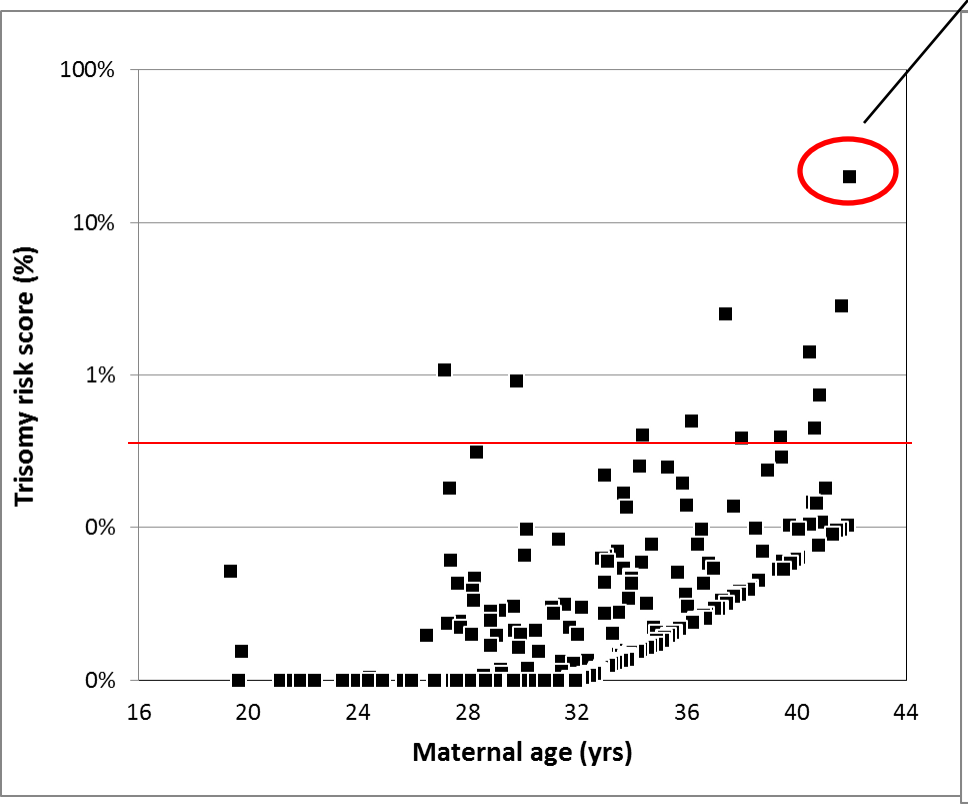
General risk: aneuploidy screening protocol



NIPT Clinical Experience (Atlanta): General Screening Population

invasive testing = normal karyotype

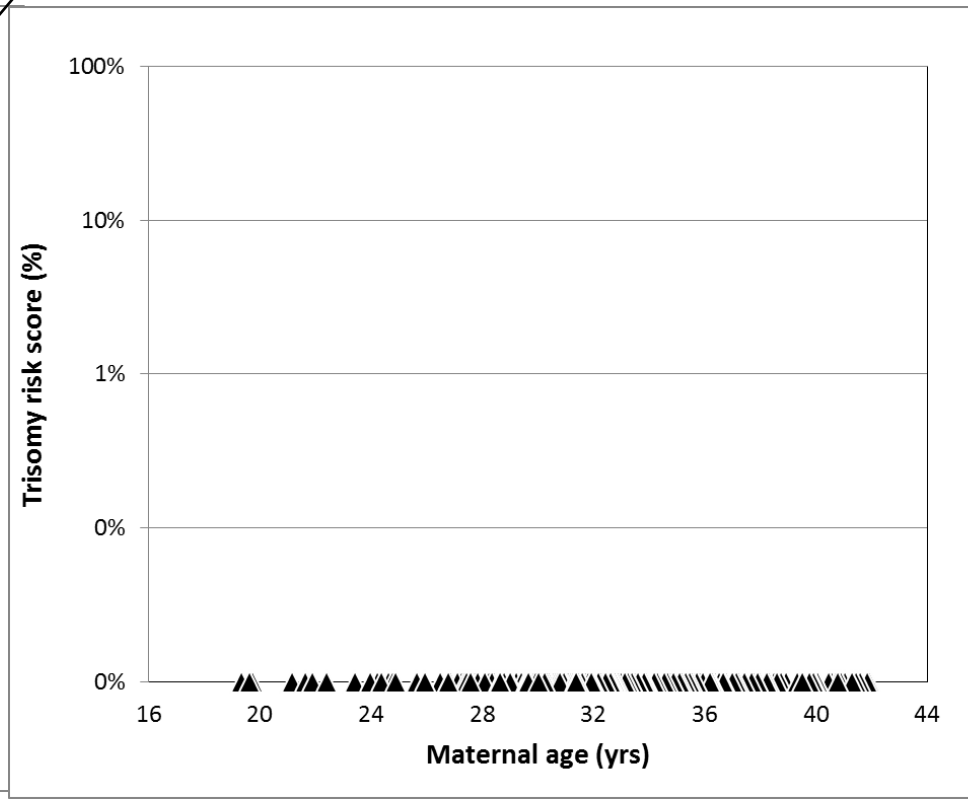
1st trimester screen risks



N = 267

12 patients (4.5%) were screen positive with FTS using a risk cut-off of 1:311

Harmony results

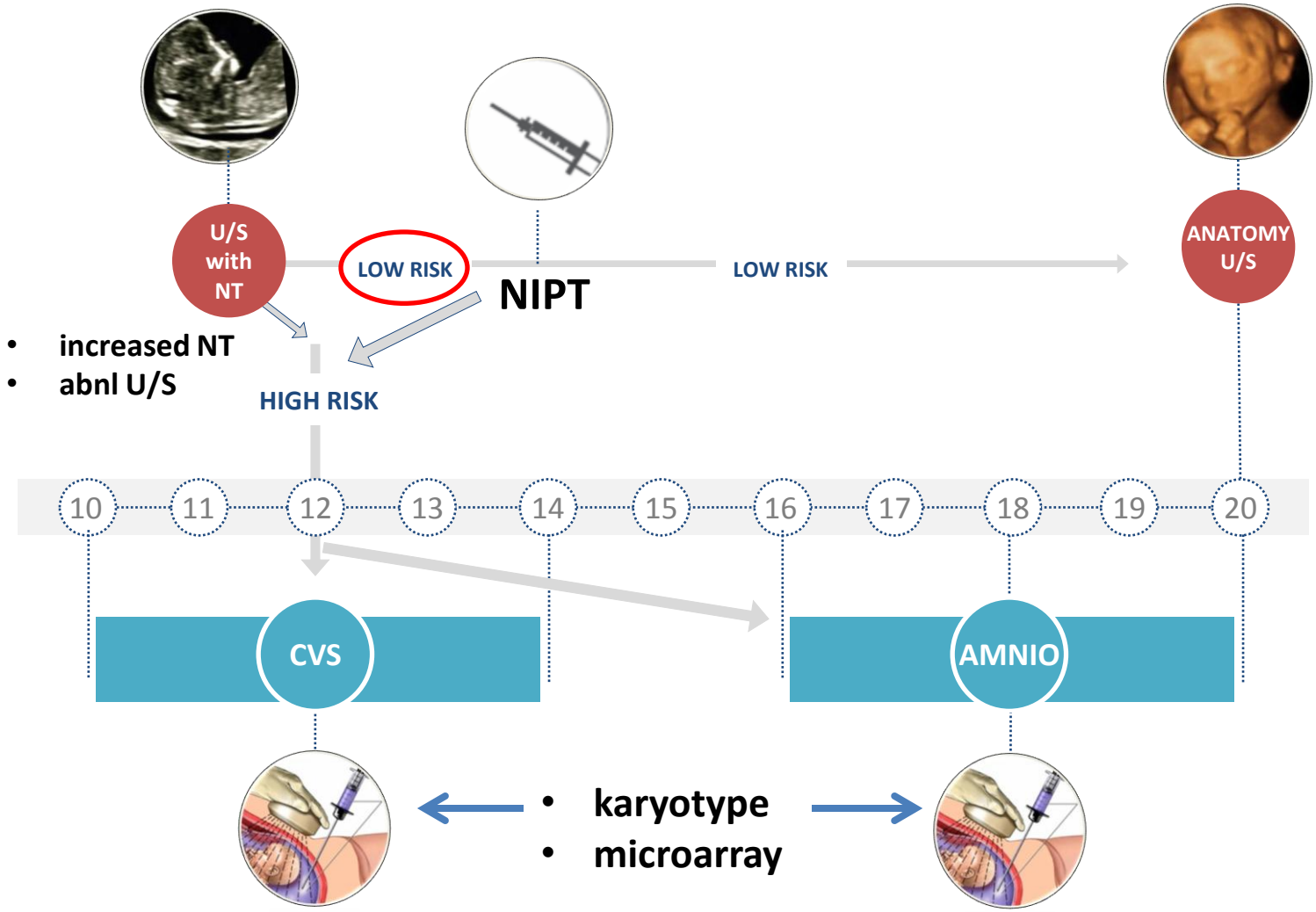


N = 287

Screen alternative to FTS: conclusions

- NIPT with cfDNA
  - substantial reduction in false positive test results as compared to 1st trimester combined screening in a general pregnancy population
  - Greater separation of **high and low** risk estimates over a range of risk cut-offs
  - Expect easier decision making regarding invasive dx
- NIPT for aneuploidy screening in the general pregnant population could help to reduce unnecessary invasive procedures and maternal anxiety

NT screen first, then NIPT for low risk



Contingent model example

FTS @ 1:100 cut-off
DR T21 = 85%

- Combined screen:
  - High risk: (eg. >1:100)
    - offer CVS +/- microarray
    - Alternative cfDNA w counseling
  - Intermediate risk (eg. > **1:3,000**)
    - approx. 20% of population
    - 14-15% of T21 cases
  - Overall detection rate T21 = 97%
  - Approx. 24% of population tested w NIPT
- Cut-off depends on resources available
- Decreased invasive testing rate (about 0.4% of pop.)
- Decreased miscarriage rate
- Retain advantages of biochemical screening

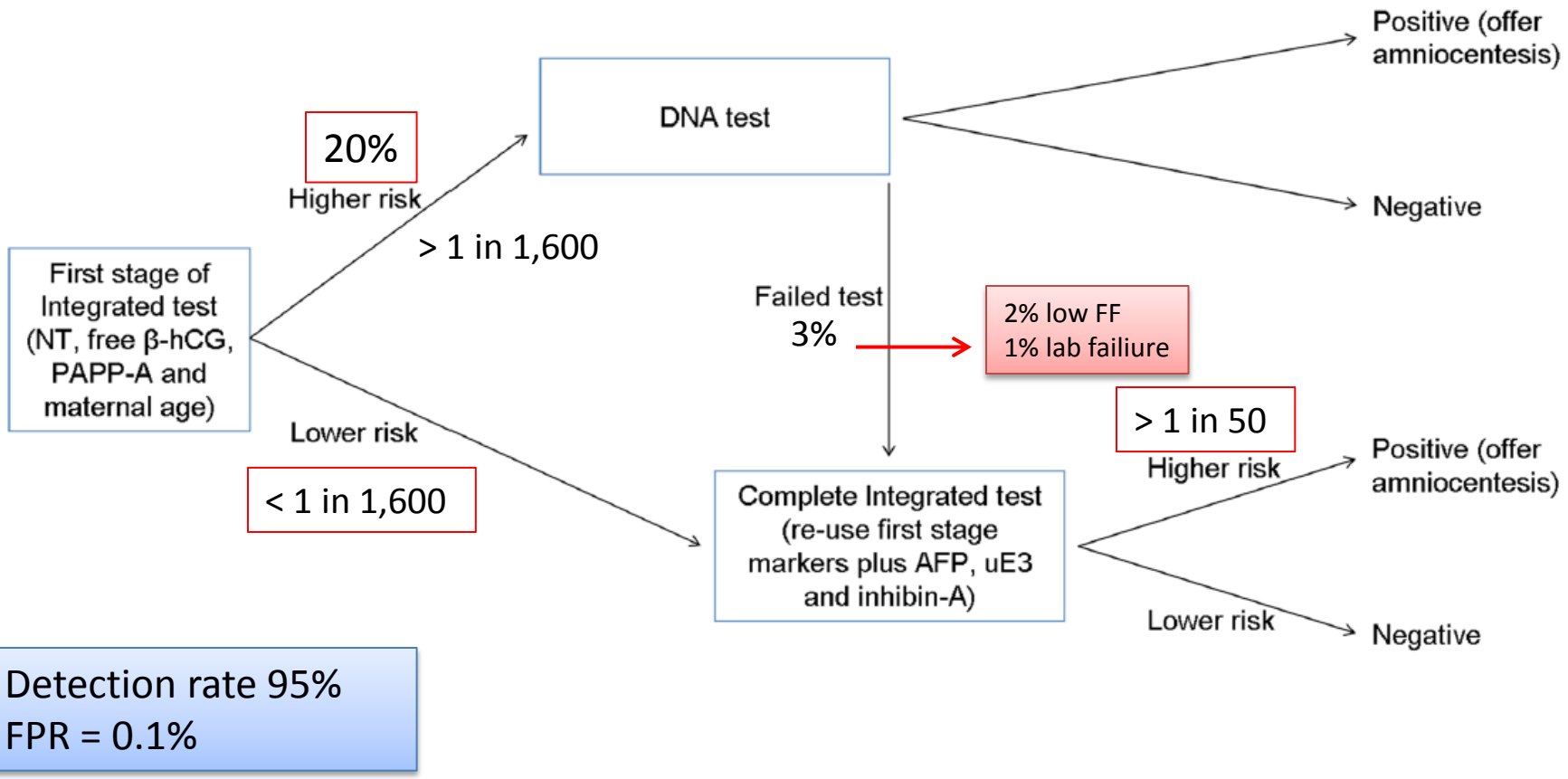
Ultrasound Obstet Gynecol. 2013 Jul;42(1):41-50. doi: 10.1002/uog.12511. Epub 2013 Jun 7.
First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM.

Contingent model:
Overall detection dependent on detection rate for first trim combined screening

	NT, PAPP-A, β-hCG		
Risk cut-off	*cfDNA (%)*	*DR (%)*	*IR (%)*
100	2.3	84.7	0.34
500	7.5	92.2	0.36
1000	12.1	94.6	0.37
1500	15.9	95.6	0.38
2000	19.2	96.3	0.39
2500	22.0	96.9	0.39
3000	24.5	96.9	0.39
3500	26.8	96.9	0.39
4000	28.9	97.3	0.40
5000	32.6	97.6	0.40
6000	35.8	98.0	0.41
7000	38.6	98.0	0.41
8000	41.2	98.0	0.41

Ultrasound Obstet Gynecol. 2013 Jul;42(1):41-50. doi: 10.1002/uog.12511. Epub 2013 Jun 7.
First-trimester contingent screening for trisomy 21 by biomarkers and maternal blood cell-free DNA testing. Nicolaides KH, Wright D, Poon LC, Syngelaki A, Gil MM.

“Sequential /contingent with NIPT”



Sequential/contigent v. cfDNA alone

TEST MODEL	DETECTION RATE	FPR
Sequential/contingent (cfDNA)	95%	0.08%
All women have DNA test (no integrated test)		
~~test failures classified as positive~~	98.6%	3.[illegible]%
test failures have Quad, risk cut-off 1 in 100	98.0%	0.39%

- Costs of program: modeled as the cost of cfDNA testing as multiple of Integ Screen cost

Expanding the test menu: is more better ?

Expanding the test menu

- Expanding the test menu to include sex chromosome aneuploidy
- What happens to False Positive Rate?
  - combined false positive rate
  - Increases with number of conditions screened

Sex Chromosome Aneuploidies

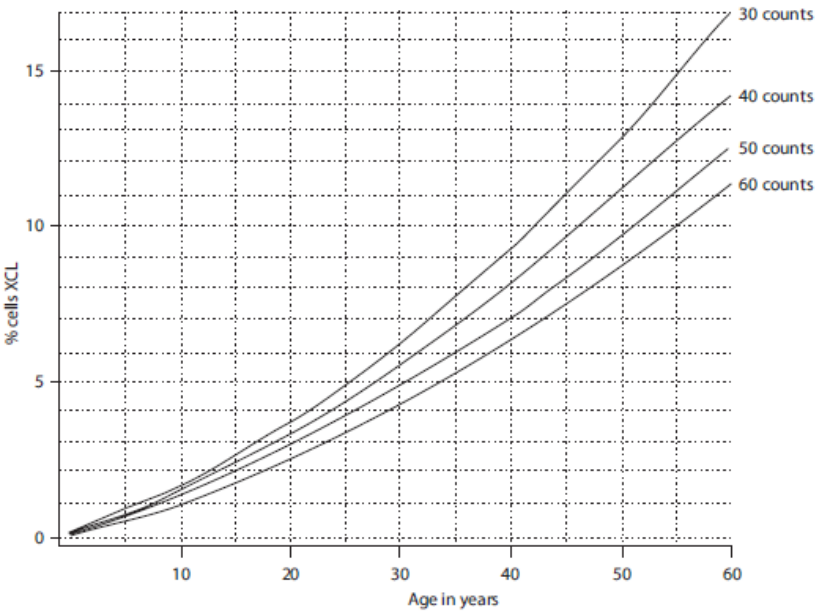
Sex aneuploidy	Birth prevalence (includes mosaics)[1]
XO	1 in 1,893 girls
XXX	1 in 947 girls
XXY	1 in 576 boys
XYY	1 in 851 boys

- Overall frequency all SCA of approx. 1 in 400-500
- Fetal mosaicism – can account for up to 50% of sex aneuploidy cases, especially monosomy X[2]
- Phenotype is highly variable
  - XXX and XYY may have no clinical manifestations

1. *Nielsen et al. Human Genetics 1991, 87: 81-83.*
2. *Thompson & Thompson Genetics in Medicine, Sixth Edition. Robert L. Nussbaum, Roderick McInnes, Willard Huntington. Saunders, 2001.*

Technical Considerations in Sex Aneuploidy Testing

- Maternal karyotype – may complicate cfDNA analysis
  - Unknown maternal karyotype
  - eg. 90% of women with 47,XX[illegible] have a third X chromosome[1]
- Mosaicism – Dual issue
  - **<u>Maternal mosaicism:</u>** Linear r[illegible] maternal age and X chromosc[illegible]
  - **<u>Fetal mosaicism:</u>**
    - 15% of cases of Klinefelter sync
    - 10% of cases of 47,XXX[1]
    - >50% of cases of Turner syndrome[4]



Russell et al, 2007
X chromosome loss and aging

1. . Orphanet J Rare Dis. 2010; 5: 8. Published online 2010 May 11
2. X chromosome loss and ageing. Cytogenet Genome Res. 2007;116(3):181-5
3. Orphanet J Rare Dis. 2006; 1: 42. Published online 2006 October 24
4. Thompson & Thompson Genetics in Medicine, Sixth Edition. Robert L. Nussbaum, Roderick McInnes, Willard Huntington. Saunders, 2001.

Guidelines and Standard of Care ?

- Prenatal screening for sex aneuploidies?
  - Generally, not standard practice
    - ? XO by NT screening
  - Currently majority of SCA detected as consequence of invasive dx for other conditions
- Professional societies have not recommended screening for sex aneuploidies

Clinical Considerations for Sex Aneuploidy Testing

- NIPT clinical performance data is limited [1,2,3,4]
  - Relatively small clinical studies
  - Test performance unknown for mosaicism
- Pre- and post-test counseling is essential
  - Phenotype for sex aneuploidies is highly variable and may result in a normal phenotype [5,6,7]

1. Jiang et al. BMC Medical Genomics 2012, 5:57
2. Bianchi et al. Obstet Gynecol 2012, 119:890
3. Zimmerman et al. Prenat Diagn 2012, 32:1;
4. http://www.verinata.com/providers/clinical-data Accessed March 5, 2013
5. Orphanet J Rare Dis. 2010; 5: 8. Published online 2010 May 11
6. Orphanet J Rare Dis. 2006; 1: 42. Published online 2006 October 24
7. Thompson & Thompson Genetics in Medicine, Sixth Edition. Robert L. Nussbaum, Roderick McInnes, Willard Huntington. Saunders, 2001.

Implementation and looking forward

Initial experiences:

- Feasibility of first line screening in 1st trimester screening population
- Low False Positive rate /decrease in invasive testing

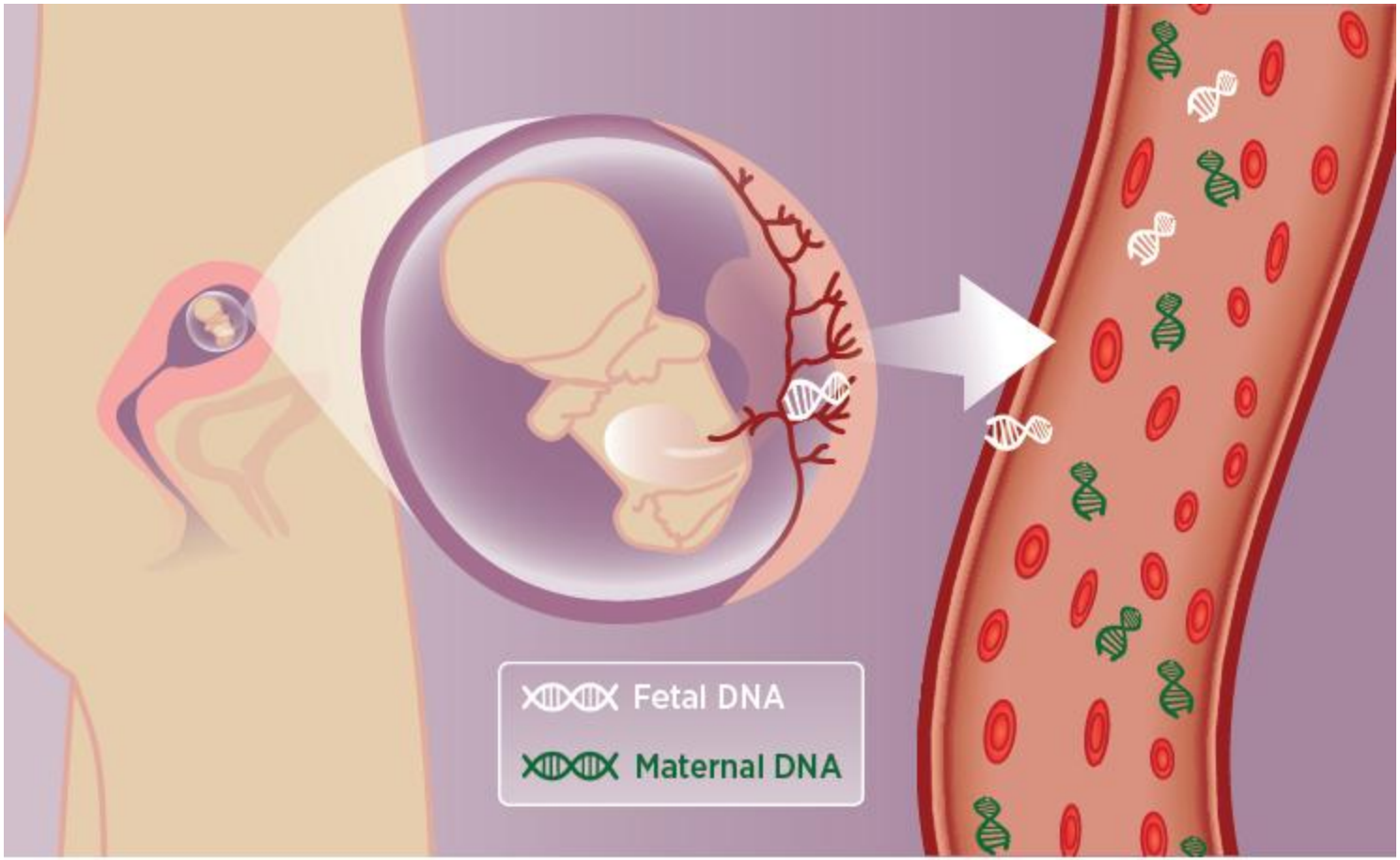
Limitations:

- Cost and resources allocated to screening program
- Counseling resources
- Physician and Patient education

The need for invasive testing:

- Invasive diagnostic testing needed with high risk cfDNA resulst and ultrasound abnormalities
- Current commercial offerings limited to 21,18,13,X,Y
- **Some patients will still desire diagnostic test:**
  - provide certainty for exclusion of: common trisomies , other aneuploidies, sub-chromosomal abnormalities (CNVs on microarray), other genetic conditions

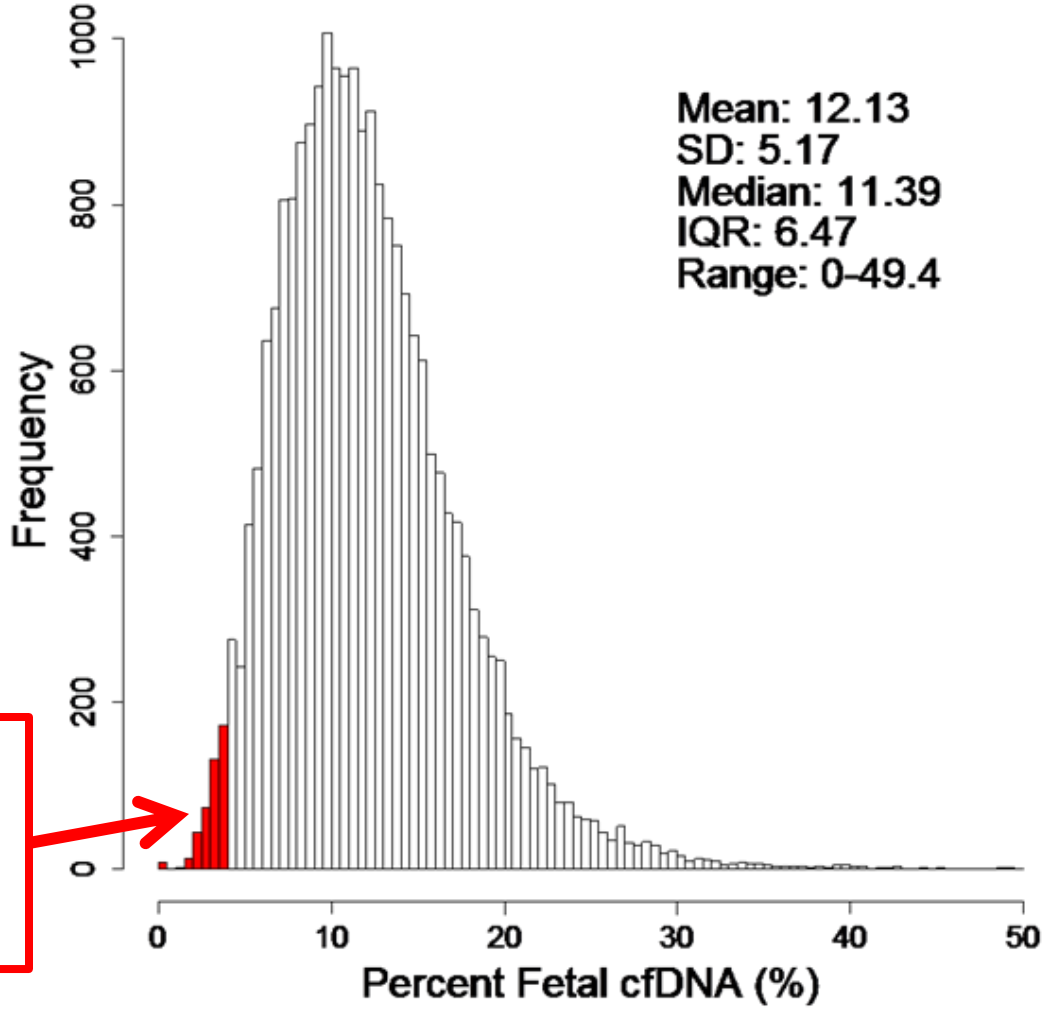
A New Era for Fetal Trisomy Detection



Genomics is changing the face of medicine !

END

Fetal Fraction – Too Low in ~2% of Women



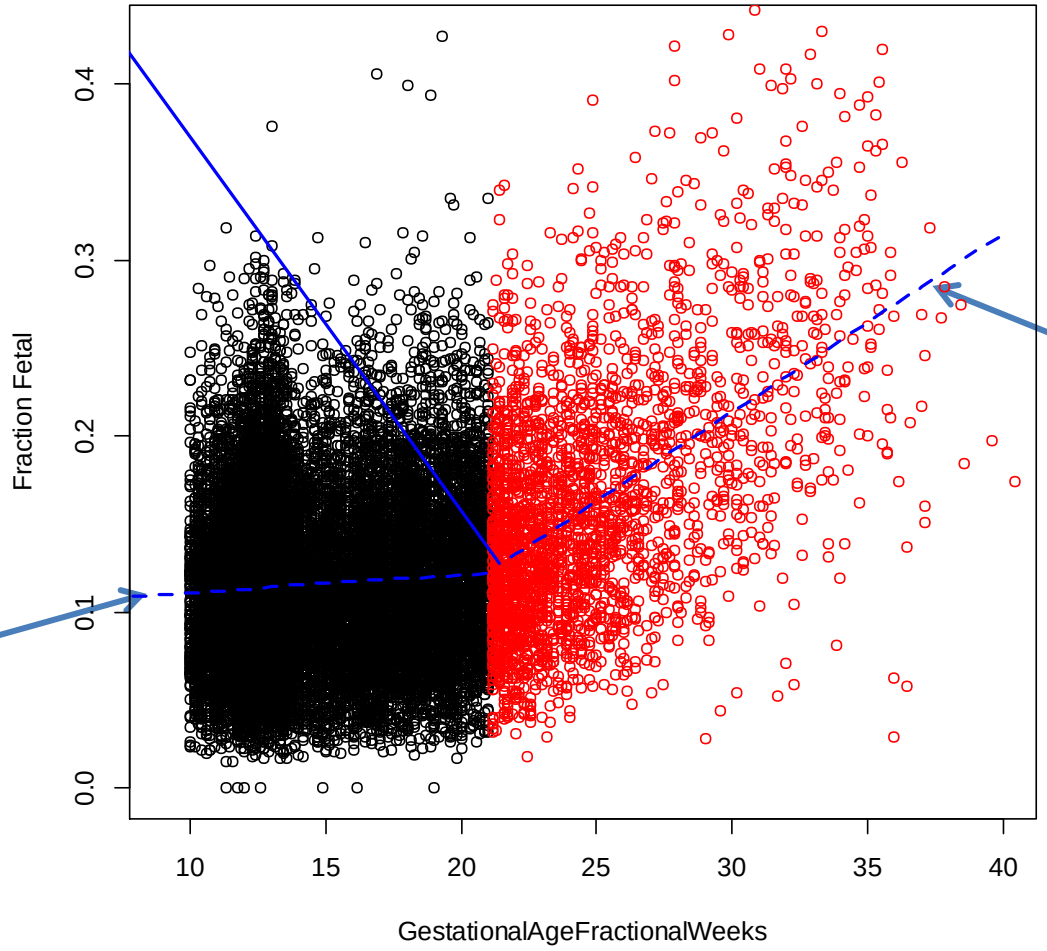
~2% (1 in 50) samples have less than 4% fetal fraction

Wang E et al, Prenat Diagn. 2013 Jul;33(7):662-6

Fetal Fraction – Gest Age Relationship

Prior to 21 wks GA:
fetal fraction increases by 0.11% per week

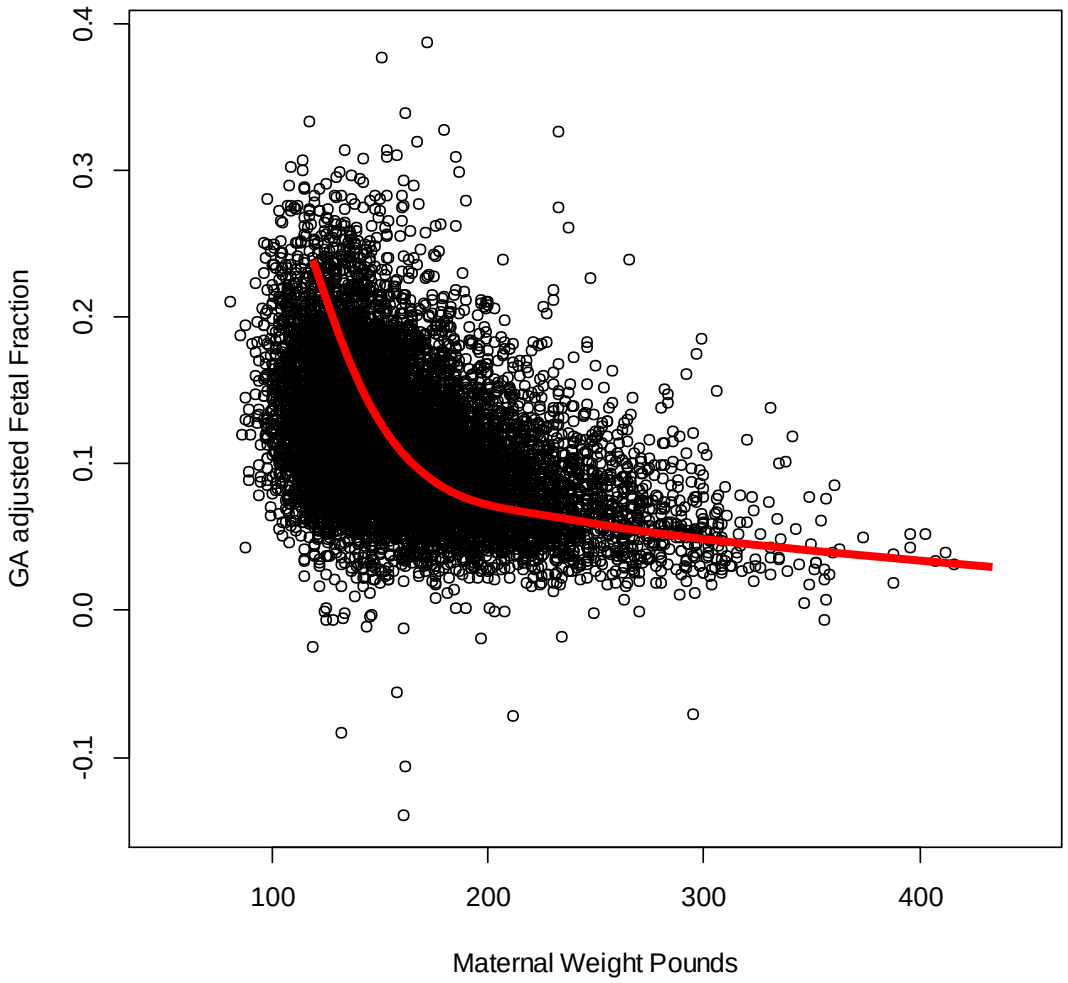
N = 22, 384



Post 21 wks GA:
fetal fraction increases by 1.1% per week

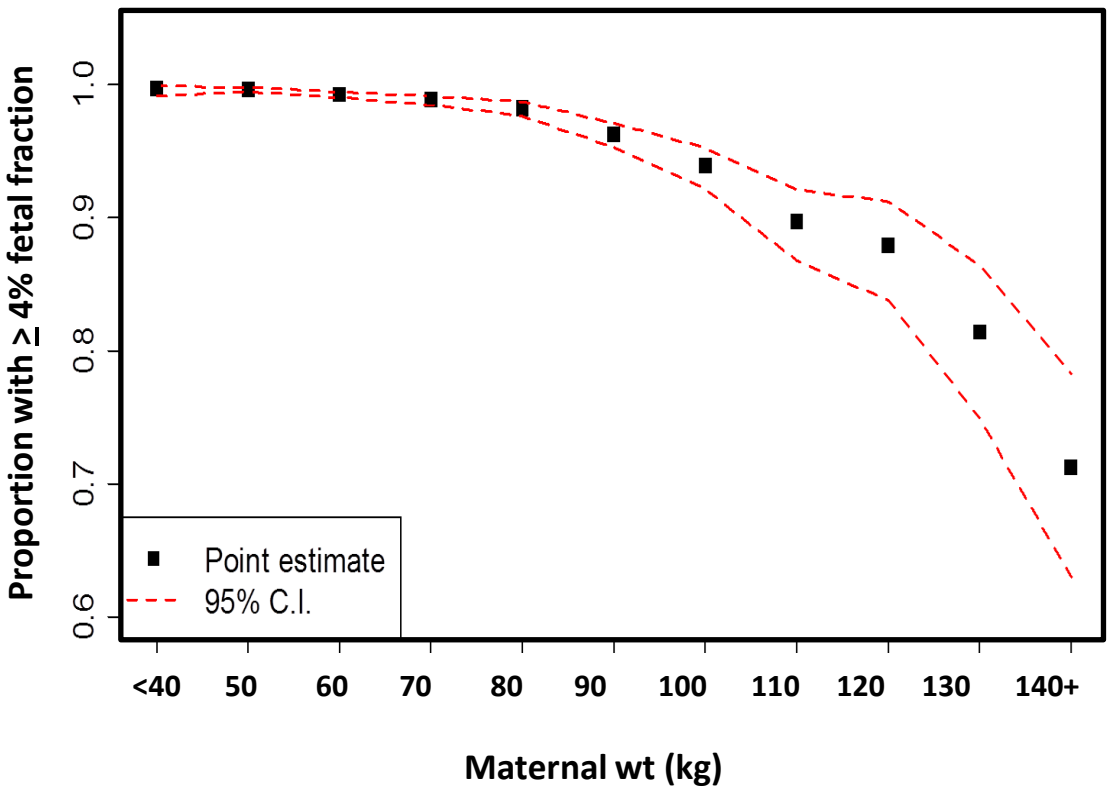
Wang et al Prenatal Diagnosis 2013 in press

Fetal cfDNA – Decreases with Higher Maternal Weight



Wang et al Prenatal Diagnosis 2013 in press

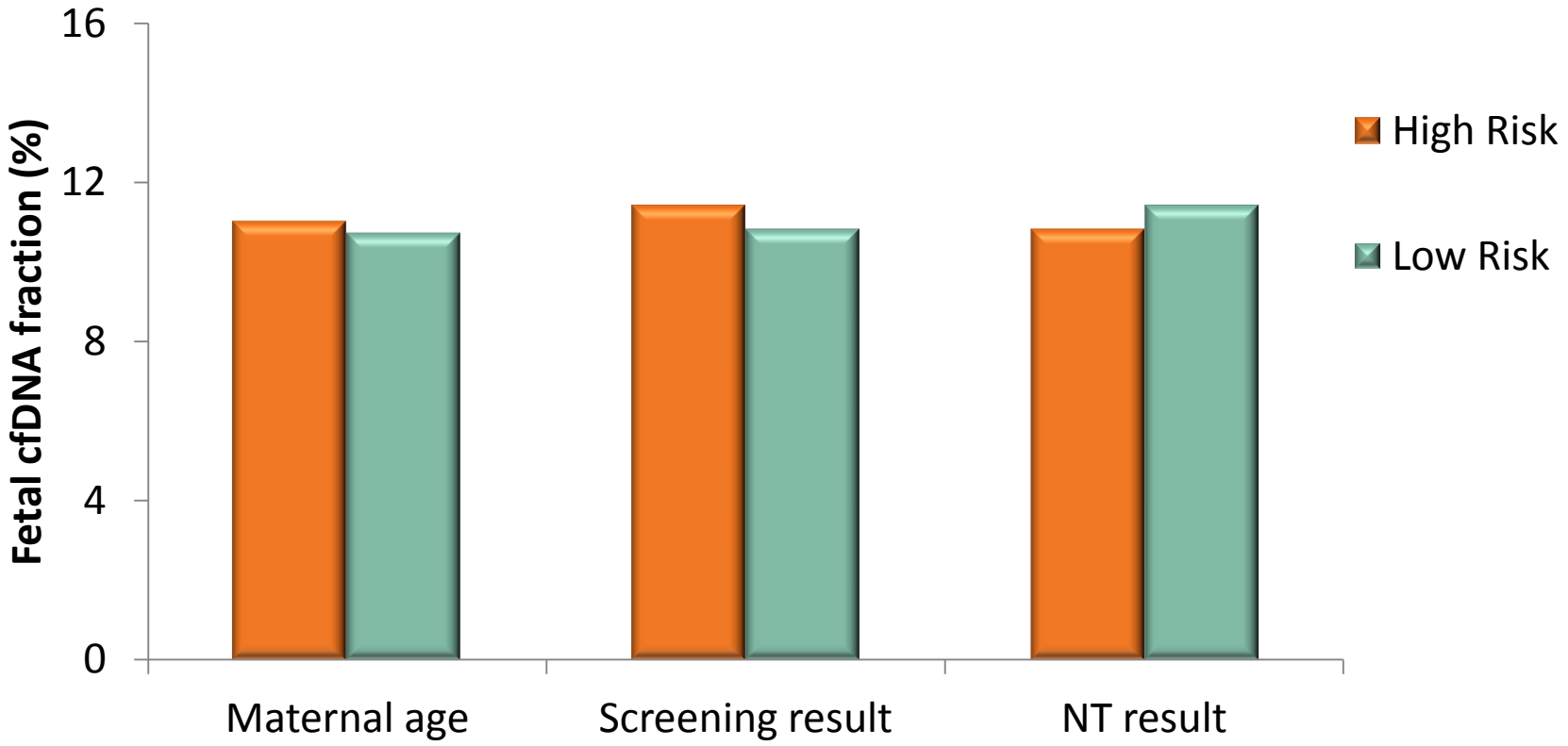
Fetal Fraction Decreases as Maternal Weight Increases



Maternal Weight (kg)	Pregnancies with ≥ 4% fetal fraction(%)
<70	>99%
≥70 & <80	99%
≥80 & <90	98%
≥90 & <100	96%
≥100 & <110	95%
≥110 & <120	90%
≥120 & <130	88%
≥130 & <140	81%
≥140	71%

Wang E et al, Prenat Diagn. 2013 Jul;33(7):662-6

Fetal Fraction – Consistent Across Pregnancy Risk Classes



"High" and "Low" risk women should both benefit from cfDNA testing

Brar H et al. *J Matern Fetal Neonatal Med,* 2012

High Risk or Average Risk?

Is NIPT only for high risk women?

What is high risk?

There is no reason to expect performance difference for general pregnant population

If sufficient cfDNA present, a result can be obtained

Simply need to account for prevalence to understand positive/ negative predictive value

- **Not a new concept in prenatal screening**

High sensitivity

Low False Positive Rates

NIPT is not diagnostic

Adding to the menu: is more better?

	Detection rate	CI	FPR
Trisomy 21	590 / 594 (99.5%)		0.1%
Trisomy 18	222 / 230 (97%)		0.1%
Trisomy 13	30 / 38 (79%)		0.1%
XX*	97.6%		0.8
XY*	99.1%		1.1
XO*	19/20 (93%)	61.5-99.9	1%
XXY	?		?
XXX	?		?
XYY	?		?
Combined false positive rate	0.3+0.8+ 1.1 + 1 = > 3.1 % + ??		

Chiu et al, 2011;
Chen et al, 2011;
Ehrich et al, 2011;
Palomaki et al, 2011;
Bianchi et al, 2012;
Sparks et al, 2012;
Ashoor et al, 2012;
Norton et al, 2012

**http://www.verinata.com/providers/provider-overview/,*

MPSS data either Published or on marketing materials

T13 – NIPT and confined placental mosaicism

Genetics in Medicine (2013)

Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism

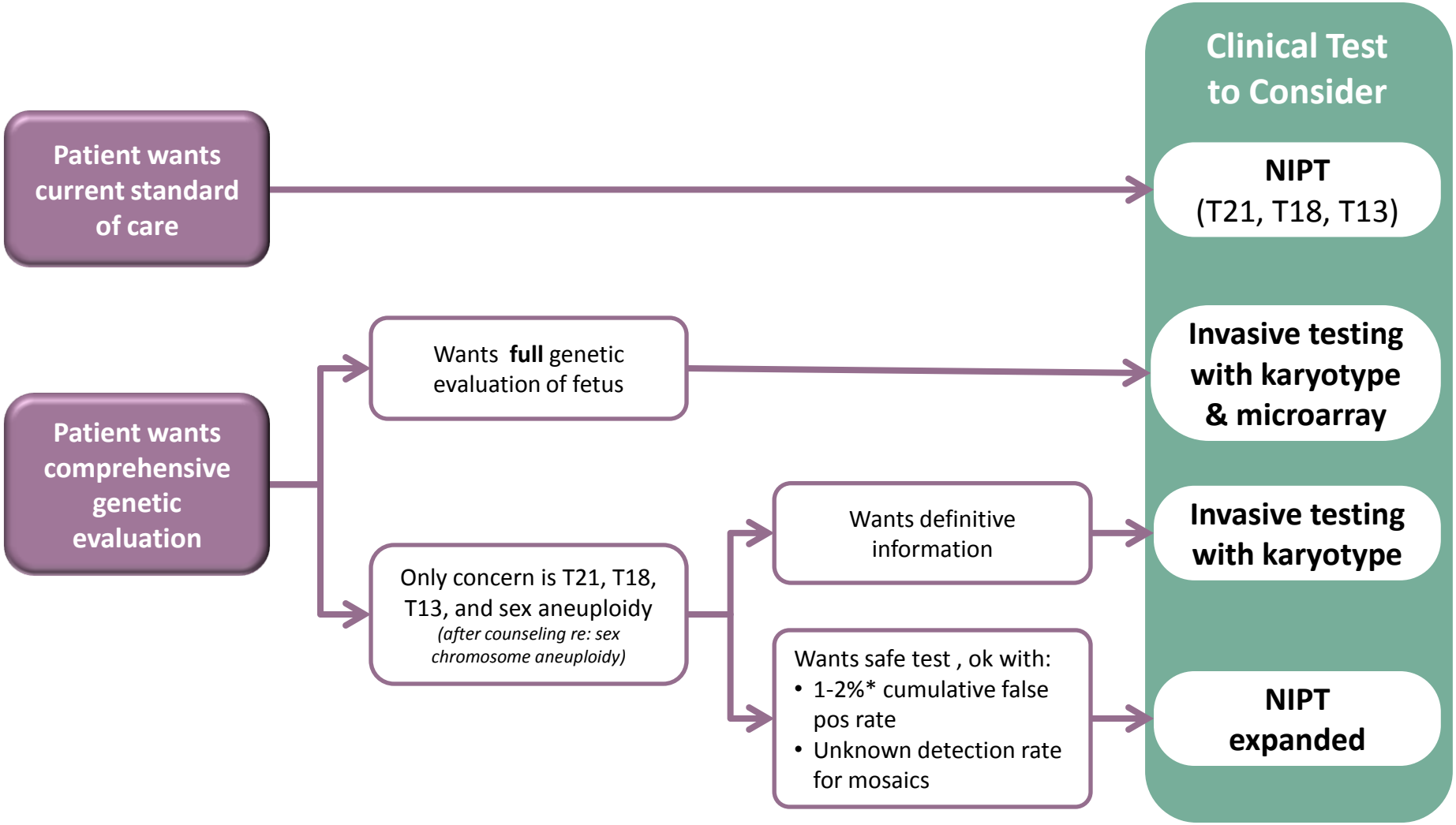
Hall AL, Drendel HM, Verbrugge JL, Reese AM, Schumacher KL, Griffith CB, Weaver DD, Abernathy MP, Litton CG, Vance GH

- **NIPT: "positive" for T13**
- **CVS: mosaic 47,XY,+13[10]/46,XY[12]**
- **Amniocentesis: 46, XY**
- **Fetus followed for intrauterine growth restriction (oligo, delivered early)**
- **Viable newborn, normal phenotype**
- **Cord blood karyotype: normal**
- **4 quadrant karyotype of placenta**
  - **2: mosaic T13**
  - **2 : normal**

Confined placental mosaicism can lead to 'discordant' cfDNA results

Confirmatory diagnostic procedure is required before further action

Adding X and Y:
Matching Clinical Tests with Expectations



First trim screen v. cfDNA alone

Women selected for reflex DNA test after Combined test	Risk cut-off for Combined test	Overall screening performance	
		Detection rate (%)	False-positive rate (%)
10%	1 in 630	90.7	0.05
20%	1 in 1600	94.1	0.07
40%	1 in 4900	96.5	0.11
60%	1 in 12000	97.4	0.15
80%	1 in 27000	97.8	0.19
90%	1 in 47000	97.9	0.20
All women have a DNA test (no Combined test):-			
test failures classified as positive		98.6	3.19
test failures have a Quadruple test, risk cut-off 1 in 100		98.0	0.29

NIPT versus Current Screening Paradigms

NIPT has many advantages over current screening methods*

Benefits:

- Simpler clinical protocol
  - Single blood test
  - Not as gestational age dependent (10-22weeks)
  - From 10 weeks on
- Higher sensitivity
- Fewer invasive tests / Safe
  - Low false positive rate
  - ~ 25 fold fewer False Positives
- Potentially earlier results

Challenges:

- For now: importance of proper informed consent
- 2-3 % test failure
  - Low fetal % cfDNA (associated with high maternal weight)
  - Assay failure
  - Follow up?
- Cost: not as low as current screening but appears to be be cost effective
- Access

*ACOG, 2012;ACMG, 2013

T18 and T13 – Mosaicism Study

American Journal of Human Genetics (1989)

Placental Mosaicism and Intrauterine Survival of Trisomies 13 and 18

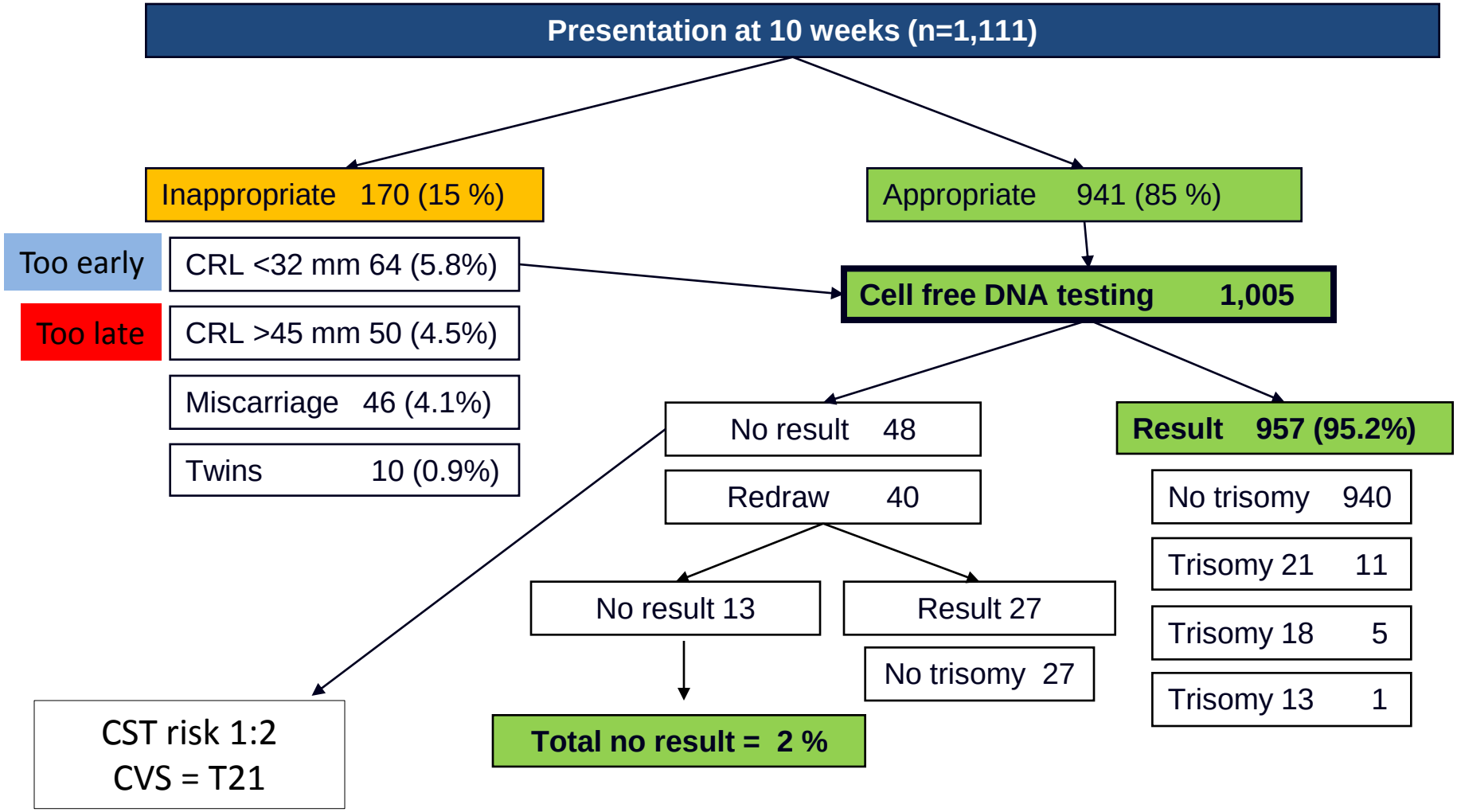
Dagmar K. Kalousek, Irene J. Barrett, and Barbara C. McGillivray

- **Methods**
  - Viable fetuses and newborns with T13, T18, and T21
  - Karyotype of cytotrophoblast, villous stroma, chorion, amnion, and cord blood
- **Results**

Fetal trisomy	% cells trisomic in cytotrophoblast	% cells trisomic in all other tissues
T13, T18 (n=14)	30% (average)	100%
T21 (n=12)	100%	100%

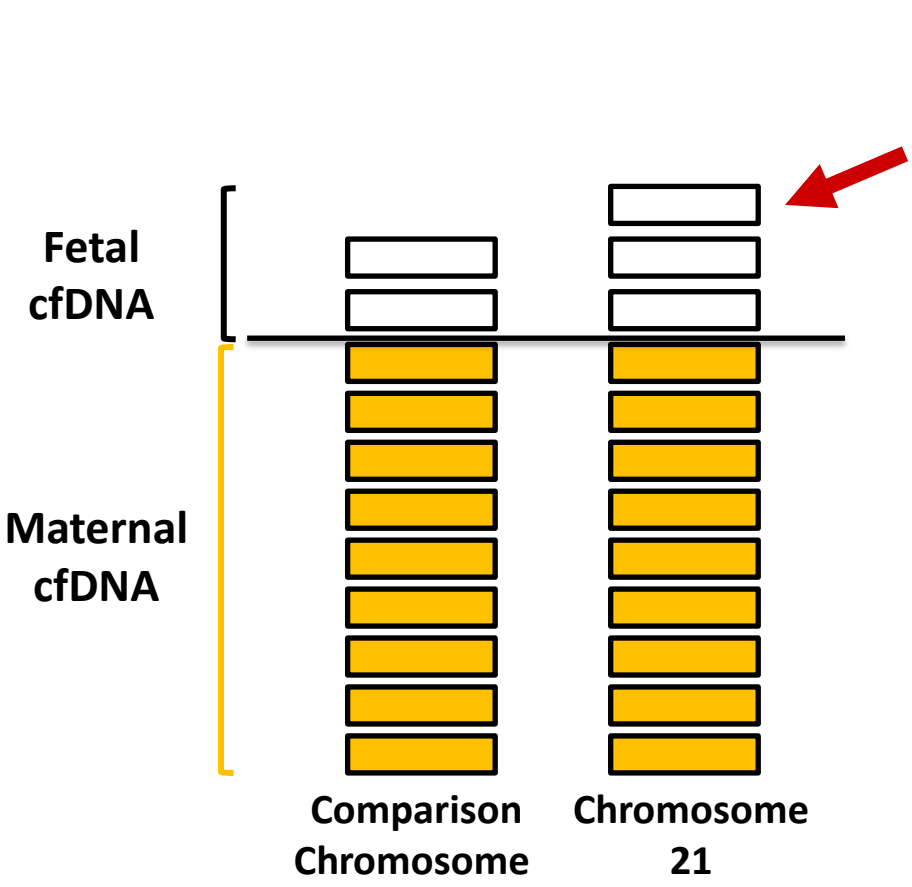
NIPT false negatives are more likely to occur in T13 and T18 due to underlying biology of fetal development

FMC/10 week protocol



Gil et al, Ultra Obstet Gyn, 2013

Importance of Fetal Fraction



Fetal Fraction	Expected ratio for Trisomy
4%	1.02
10%	1.05
20%	1.10
40%	1.20

* The higher the fetal fraction, the easier it becomes to detect aneuploidy

Importance of Fetal Fraction

- Approx. 4% or greater fetal fraction is required for reliable testing with cfDNA analysis
- Fetal cfDNA measurement is a required basic laboratory quality metric (not measured by all labs)
- Factors leading to low fetal fraction of cfDNA (<4%)
  - Maternal weight
  - Gestational age
  - Suboptimal blood collection and shipping
  - Other biological and environmental factors

PRENATAL DIAGNOSIS

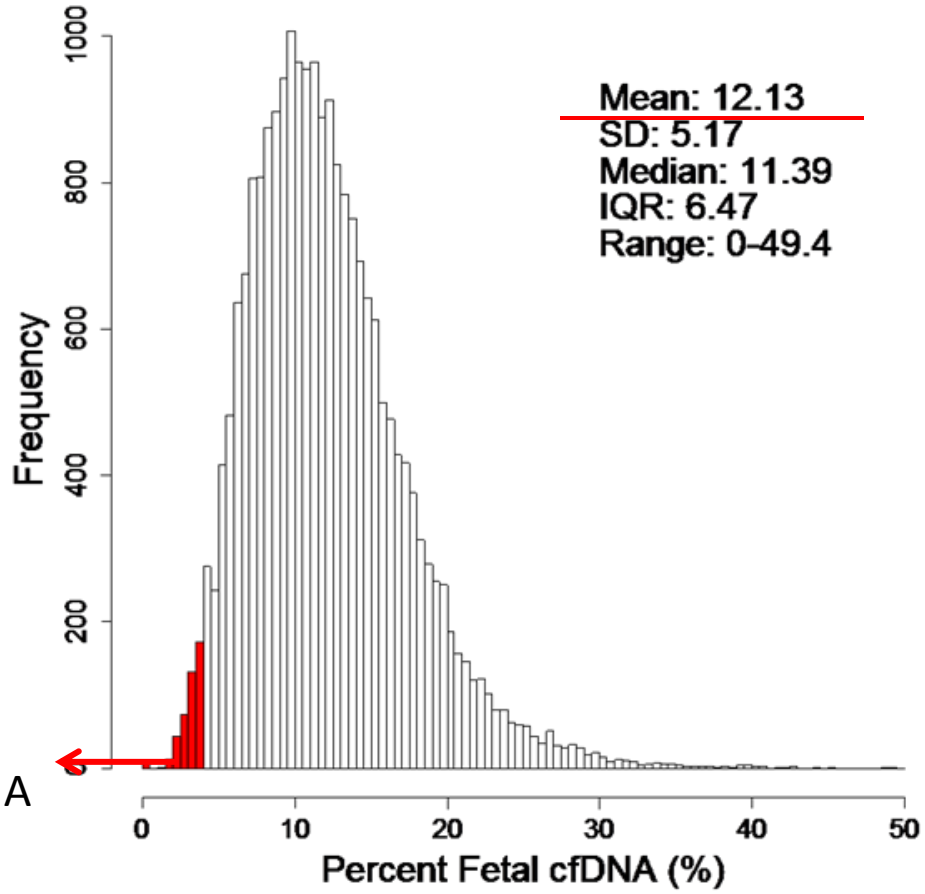
Gestational Age and Maternal Weight Effects on Fetal cfDNA in Maternal Plasma

Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A,
Prenat Diagn. 2013 Apr 2:1-5. doi: 10.1002/pd.4119. [Epub ahead of print]

Study of 22,384 commercial samples

Fetal fraction of cfDNA in maternal plasma

22,384 samples



2% samples
< 4% fetal cfDNA

Wang et al Prenatal Diagnosis 2013 in press

Fetal Fraction decreases with increasing maternal weight

N = 22,384 samples

Maternal Weight Bin (kg)	n	Pregnancies with ≥4% fetal cell-free DNA (%)
<50	809	99.8
≥50 & <60	4825	99.6
≥60 & <70	6224	99.2
≥70 & <80	4313	98.8
≥80 & <90	2574	98.2
≥90 & <100	1608	96.3
≥100 & <110	921	93.9
≥110 & < 120	508	89.8
≥120 & <130	298	87.9
≥130 & <140	172	81.4
≥140	132	71.2

100 kg = 220 lbs.

140 kg = 308 lbs.

Wang et al, Prenat Diagn. 2013 Apr 2:1-5. doi: 10.1002/pd.4119.

Professional guidelines / Payer Policy

Broad support of NIPT as most effective screening test for aneuploidy

High Risk | **Not restricted** | **High and Low RIsk**

ACOG/SMFM

- published December 2012 (written summer/fall 2012)
- No studies in low-risk women considered in this opinion

ACMG statement (Feb 2013)

- **Screening test**

Non Invasive Prenatal Screening

- **Advantage: high detection rates / low FP rates v. serum screening**
- **recommended pre-test and post-test counseling**
  - **false positives and false negatives**

BLUE CROSS / BLUE SHIELD

- **advanced screen for trisomy 21 in high and average risk pregnant women**
- **Confirmatory invasive testing**

NIPT – Economic Model

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE

ORIGINAL ARTICLE

Clinical utility and cost of non-invasive prenatal testing with cfDNA analysis in high-risk women based on a US population

Ken Song[1], Thomas J. Musci[1], and Aaron B. Caughey[2]

Key clinical outcomes		Findings
* Trisomy detection	→	* NIPT detects 28-43% more cases
* Invasive procedures	→	* NIPT reduces by >95%
* Euploid fetal loss	→	* NIPT reduces by >99%

NIPT at less than $1,000 was cost savings

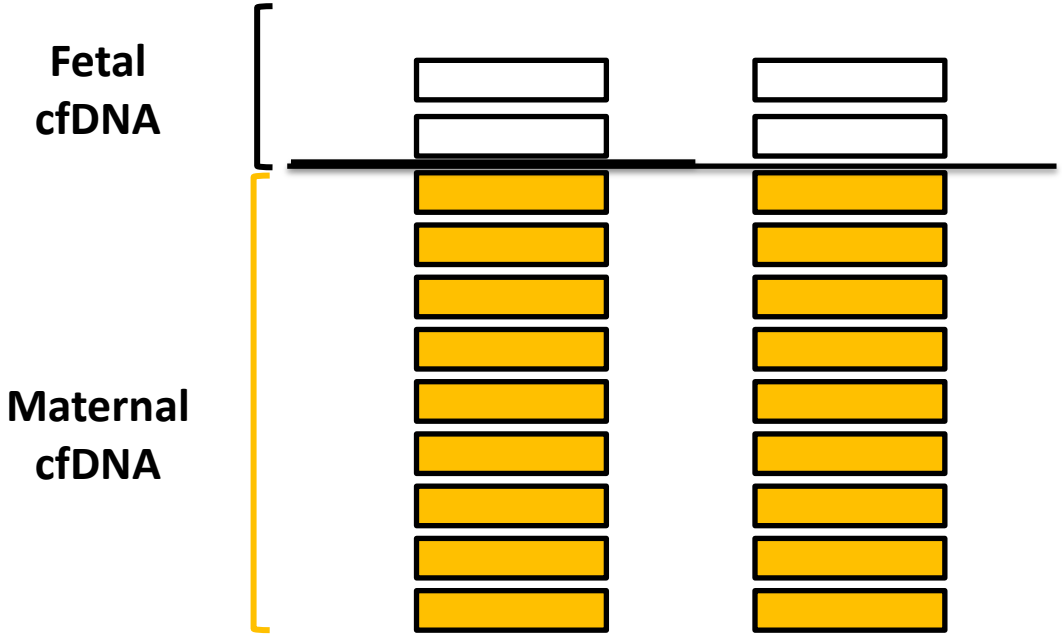
NIPT Clinical Experience (Atlanta): General Screening Population

- **Harmony alternative to 1st trim combined screening**

Characteristic	Values
Maternal age, yrs, mean ± SD (range)	32.3 ± 4.7 (17.8-42.0)
Gestational age, wks, mean ± SD (range)	13.0 ± 1.5 (10.1-20.7)
Fetal fraction, %, mean + SD (range)	12.4 ± 4.5 (2.9-37.6)

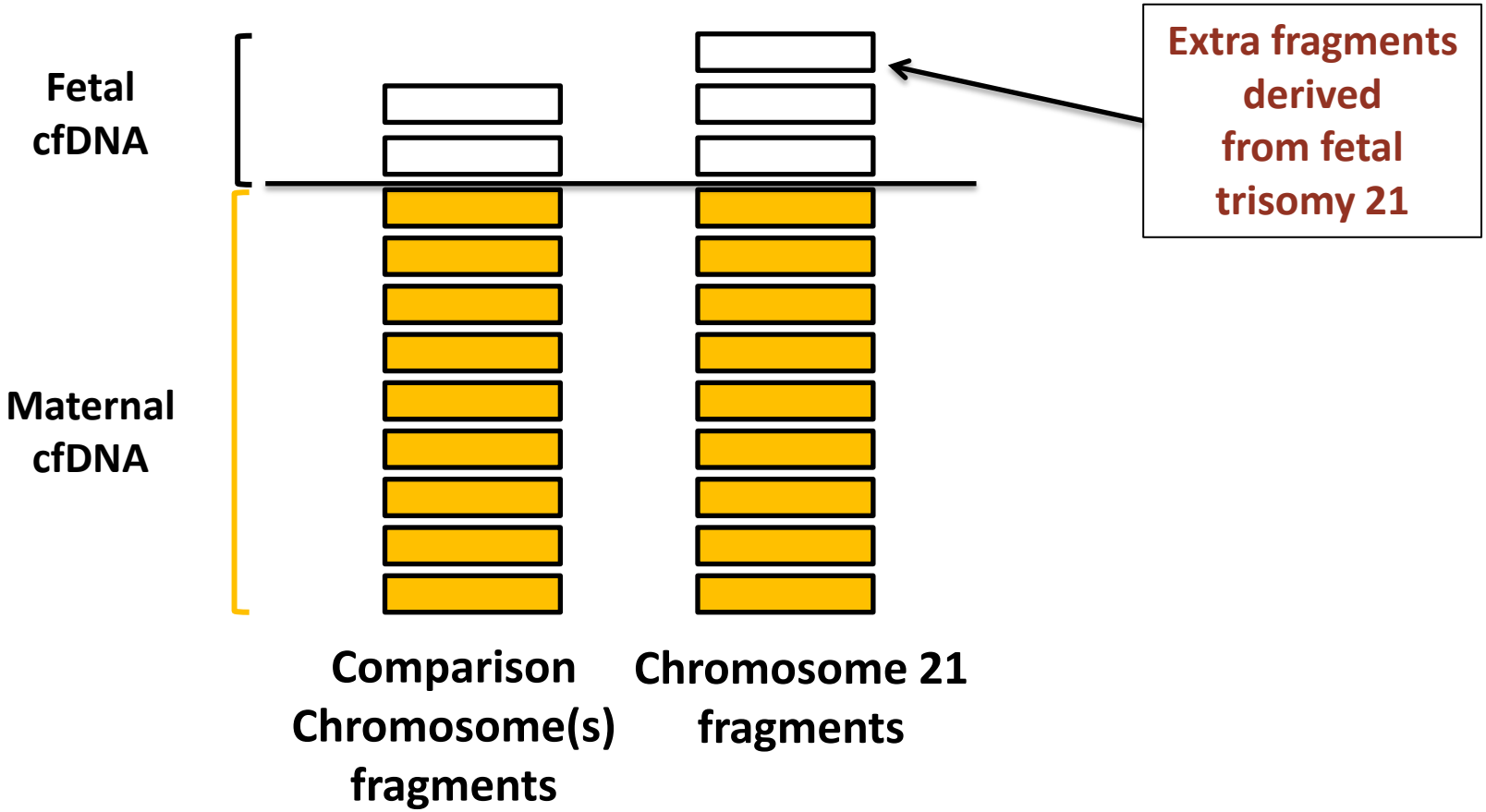
N = 289
1 sample delay in transit (>5 days)
98.6% (284 of 288) received result

Chromosome counting with cfDNA



Total cfDNA fragments

Fetal Trisomy Detection With cfDNA



* The overabundance of chromosome 21 cfDNA fragments in trisomy 21, although small, can be measured with DNA sequencing

Implementation and looking forward

- Initial experience :
  - Feasibility of first line screening in 1st trimester screening population
  - Low False Positive rate and decrease in invasive testing
- Invasive diagnostic testing needed with high risk and ultrasound abnormalities (including increased NT measurement)
  - Current commercial offerings limited to 21,18,13,X,Y
- Limitations to implementation:
  - Cost and resources allocated to screening program
  - Counseling resources
  - Physician and Patient education
- **Some patients will still desire diagnostic test:**
  - to provide certainty for exclusion of:
    - common trisomies ,
    - other aneuploidies,
    - sub-chromosomal abnormalities (copy number variants on microarray)