NC NEXUS
FDA Teleconference

5/2/14

Three Groups of Children to be Studied

- Cohort 1: children ages 0-5 years with one of four conditions identified by NBS
  - PKU
  - MCADD
  - CF
  - Hearing loss
- Cohort 2: Children with rare disorders not detected by NBS
  - Primary ciliary dyskinesia
  - Mucopolysaccharidoses
  - Wilson disease
  - Adrenoleukodystrophy
  - Sex chromosome variations
- Cohort 3: Well-child group, prenatal recruitment

Definitions

- WES = whole exome sequencing with focused informatics analysis
- NGS-NBS = next generation sequencing newborn screen; a sequencing-based assay to augment current newborn screening by identifying medically actionable conditions that manifest in childhood
- VUS = variant of uncertain significance

Basic Design

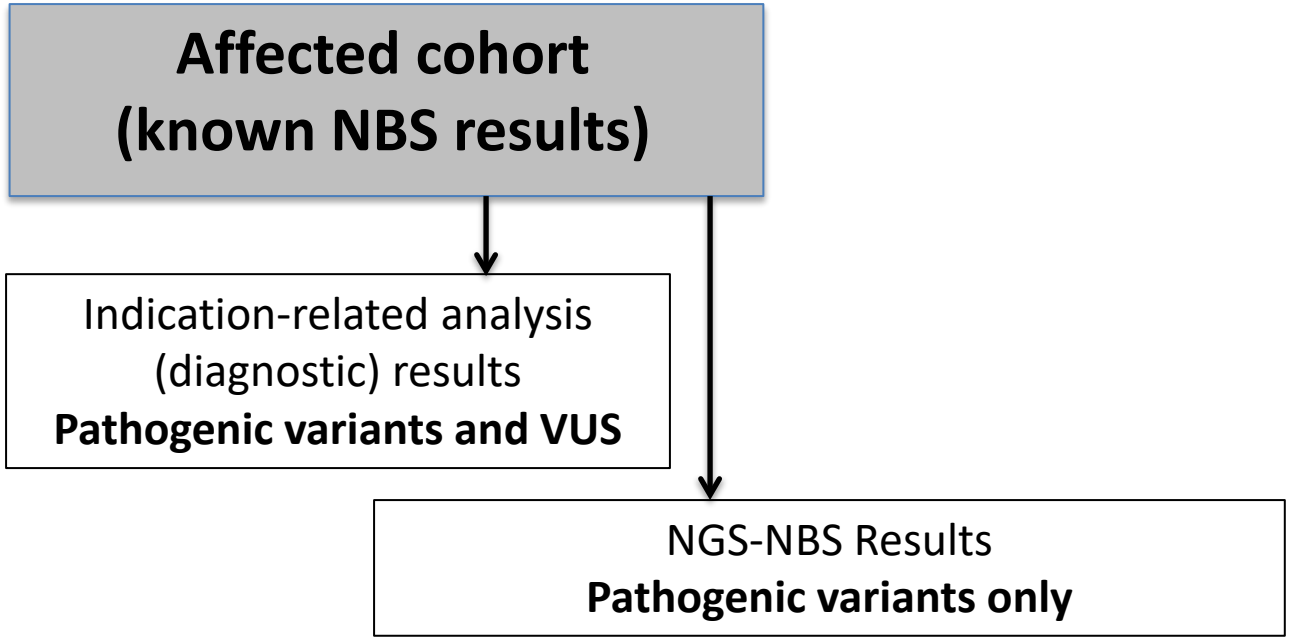
- Invite families from the three groups to participate in a study involving WES
- Determine initial acceptance rate, reasons for accepting or declining, factors associated with decisions
- Target acceptors – 200 total from the “diagnosed” cohorts and 200 total well-child
- For those who accept WES, randomly assign to NGS-NBS only or to NGS-NBS with decision whether or not to obtain additional information
- Study choices and consequences

Affected cohort (known NBS results)

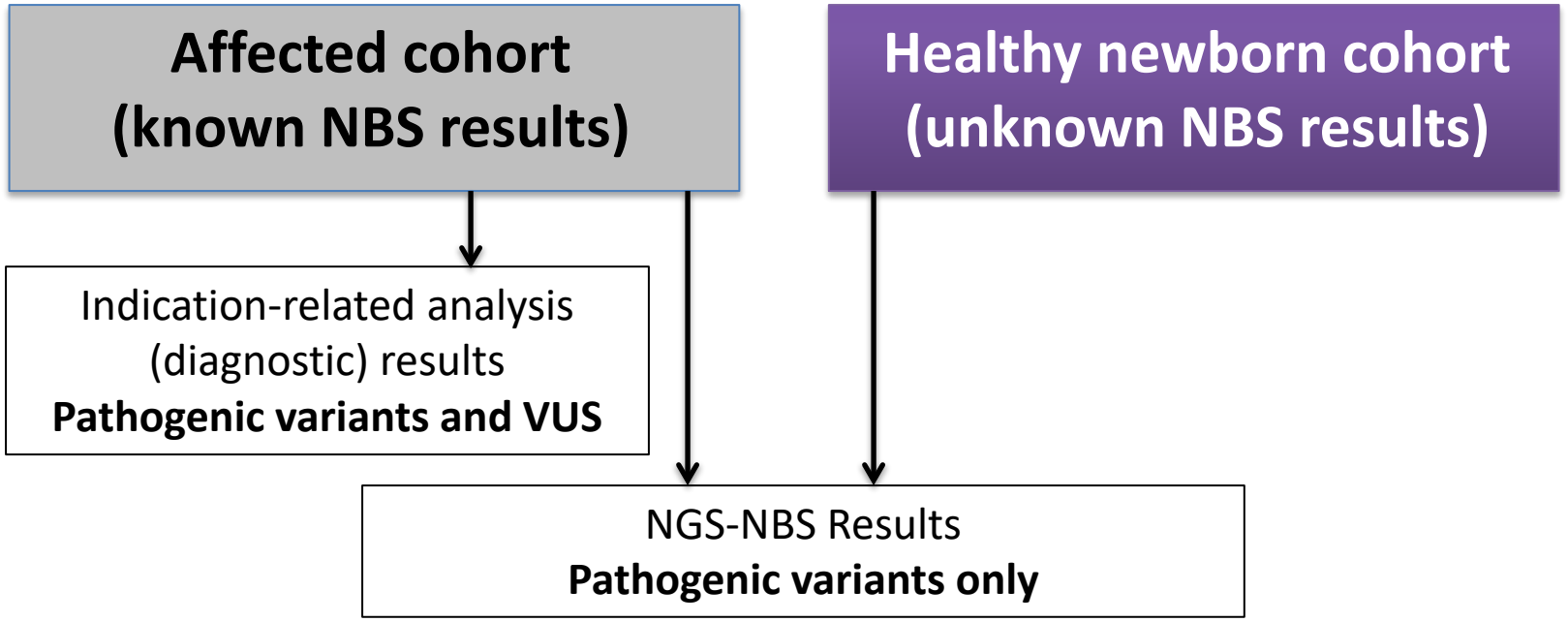
↓

Indication-related analysis (diagnostic) results
Pathogenic variants and VUS

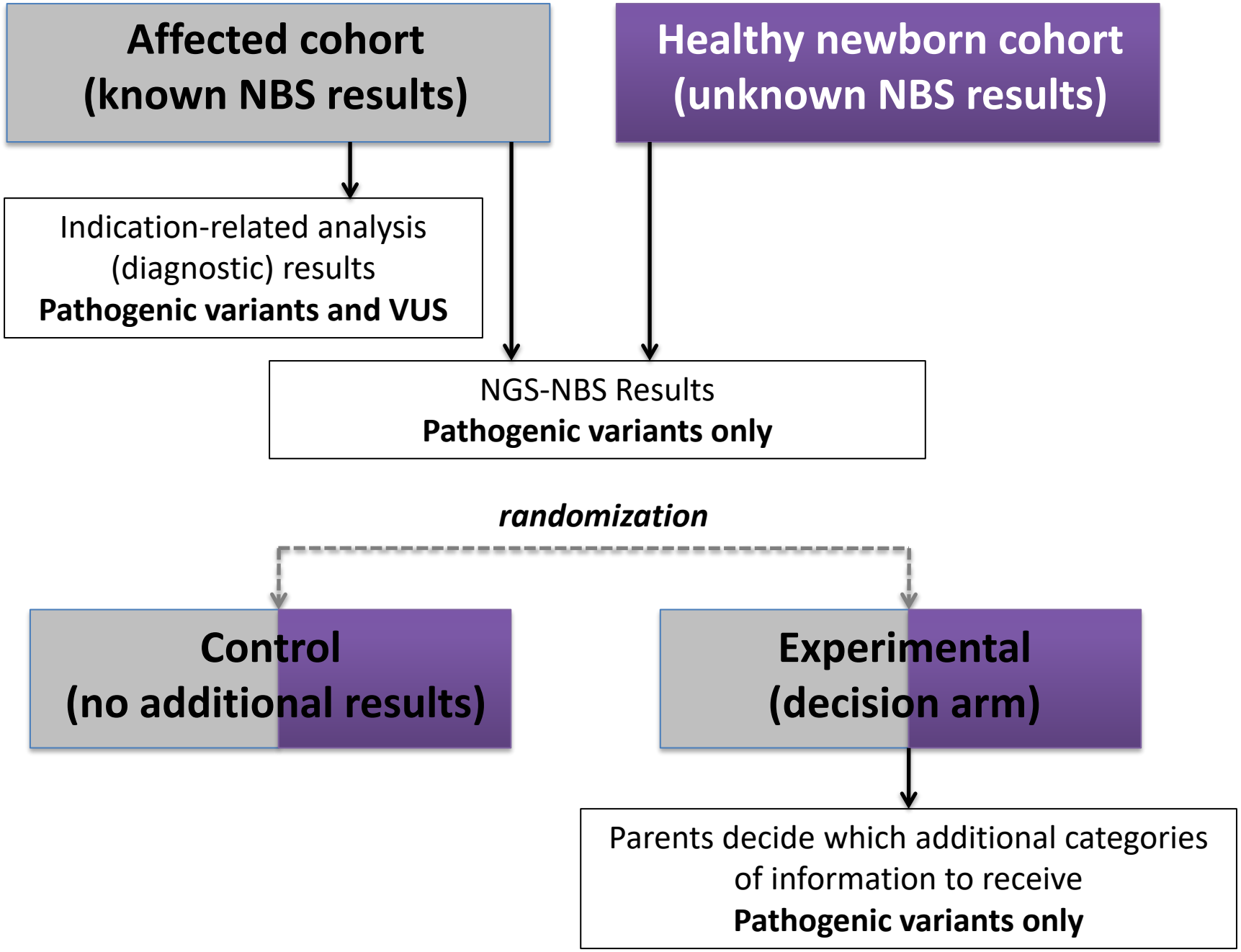
We expect a high rate of positive findings in the indication-related analysis because participants in the affected cohort have known disorders that should be amenable to detection with next-generation sequencing



We expect a very low rate of NGS-NBS positive findings in the affected cohort other than the indication-related (diagnostic) results



We expect a very low rate of NGS-NBS positive findings in the healthy newborn cohort (1-3%), so the majority of participants will receive "negative" results

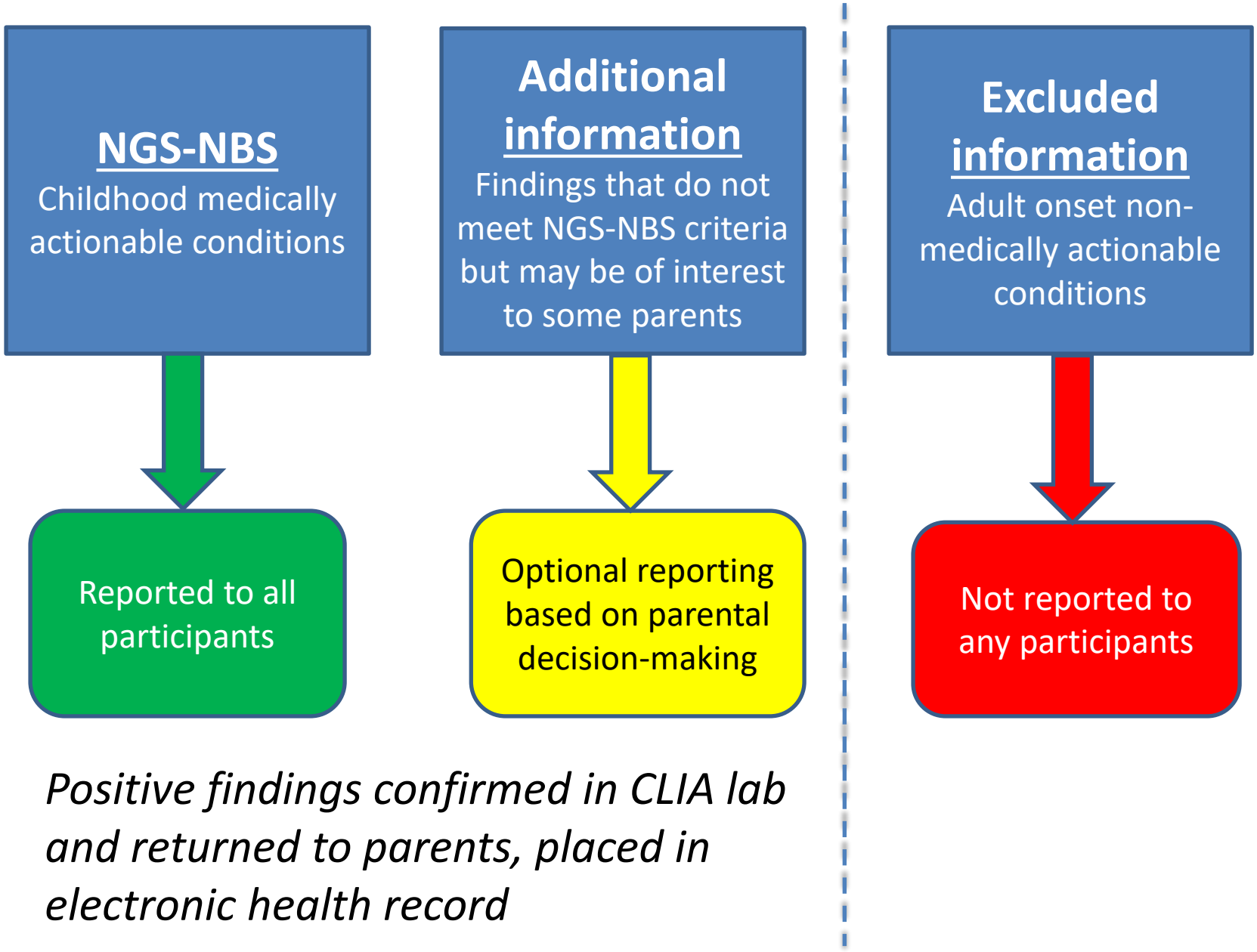

Affected cohort
(known NBS results)
Healthy newborn cohort
(unknown NBS results)
Indication-related analysis
(diagnostic) results
Pathogenic variants and VUS
NGS-NBS Results
Pathogenic variants only
randomization
Control
(no additional results)
Experimental
(decision arm)
Parents decide which additional categories
of information to receive
Pathogenic variants only

Positive result confirmation/reporting

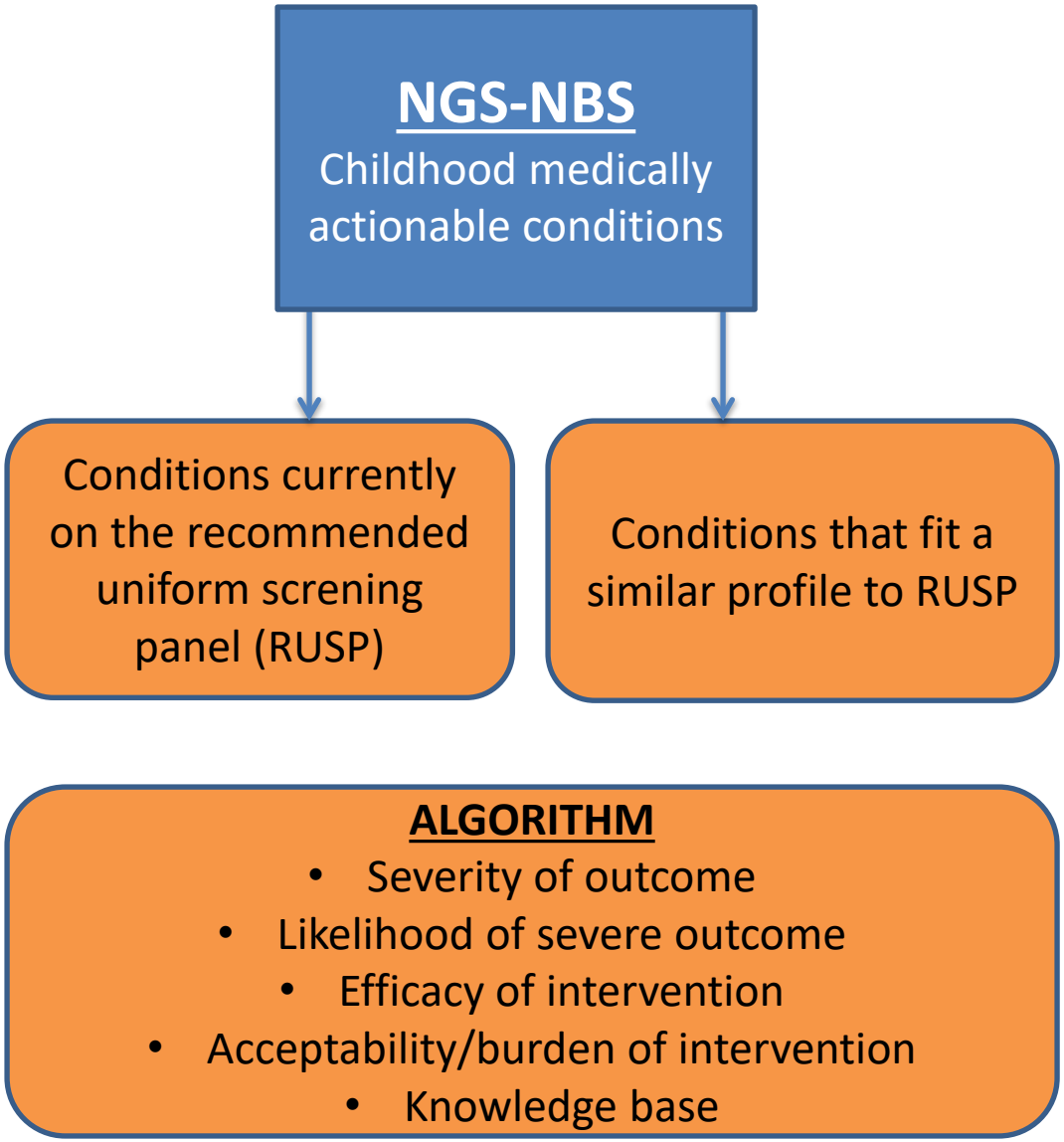
- All positive findings will be confirmed in the CLIA lab
- We will use the most clinically appropriate test
  - Sanger sequencing appropriate for most NGS findings (substitutions, small insertions/deletions)
  - FISH or microarray would be more suitable for large deletions/duplications
  - Karyotype to confirm suspected aneuploidy
- CLIA results will be generated and signed out by ABMG certified molecular geneticists or cytogeneticists
  - Able to be entered into medical record
  - IRB has not yet weighed in on need for separate consent to place results in medical record

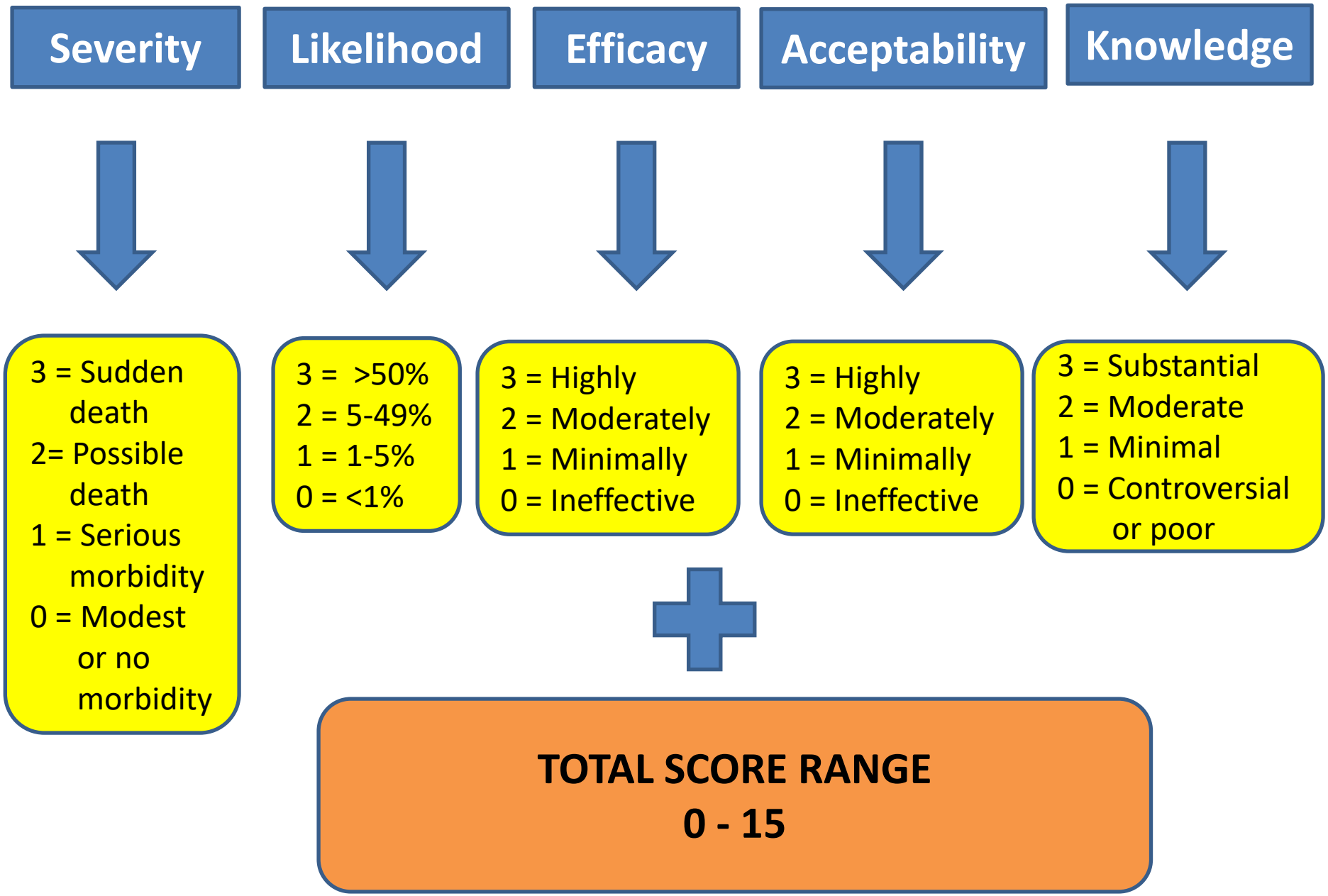
Reporting negative results

- We propose to provide negative results as a “research report”
  - Not placed in medical record
- Possible types of information that could be included in the “research report”:
  - Coverage metrics
  - Genes analyzed
  - Aggregate information about numbers of variants
  - Disclaimer regarding limitations of WES
- Is this information considered to add “risk”?



Positive findings confirmed in CLIA lab and returned to parents, placed in electronic health record


NGS-NBS
Childhood medically actionable conditions
Conditions currently on the recommended uniform screning panel (RUSP)
Conditions that fit a similar profile to RUSP
ALGORITHM
• Severity of outcome
• Likelihood of severe outcome
• Efficacy of intervention
• Acceptability/burden of intervention
• Knowledge base


Severity
Likelihood
Efficacy
Acceptability
Knowledge
3 = Sudden death
2= Possible death
1 = Serious morbidity
0 = Modest or no morbidity
3 = >50%
2 = 5-49%
1 = 1-5%
0 = <1%
3 = Highly
2 = Moderately
1 = Minimally
0 = Ineffective
3 = Highly
2 = Moderately
1 = Minimally
0 = Ineffective
3 = Substantial
2 = Moderate
1 = Minimal
0 = Controversial or poor
TOTAL SCORE RANGE
0 - 15

Example: PAH (Phenylketonuria)

- Severity: intellectual disability = 1
- Likelihood: highly penetrant = 3
- Effectiveness of intervention: diet = 3
- Acceptability of intervention: diet = 2
- Knowledge base: high = 3

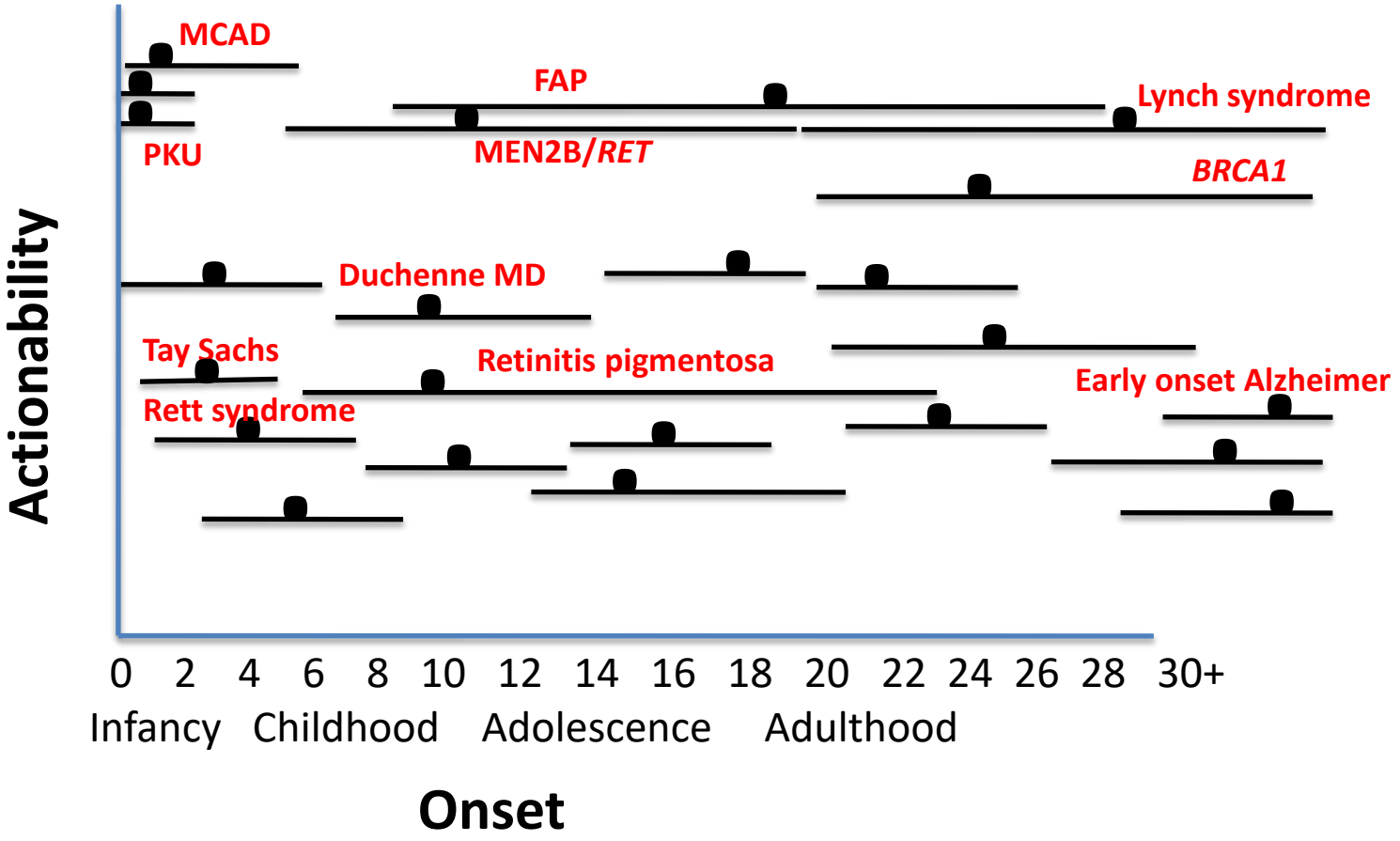
- Total score of 12

Example: APC (Familial adenomatous polyposis)

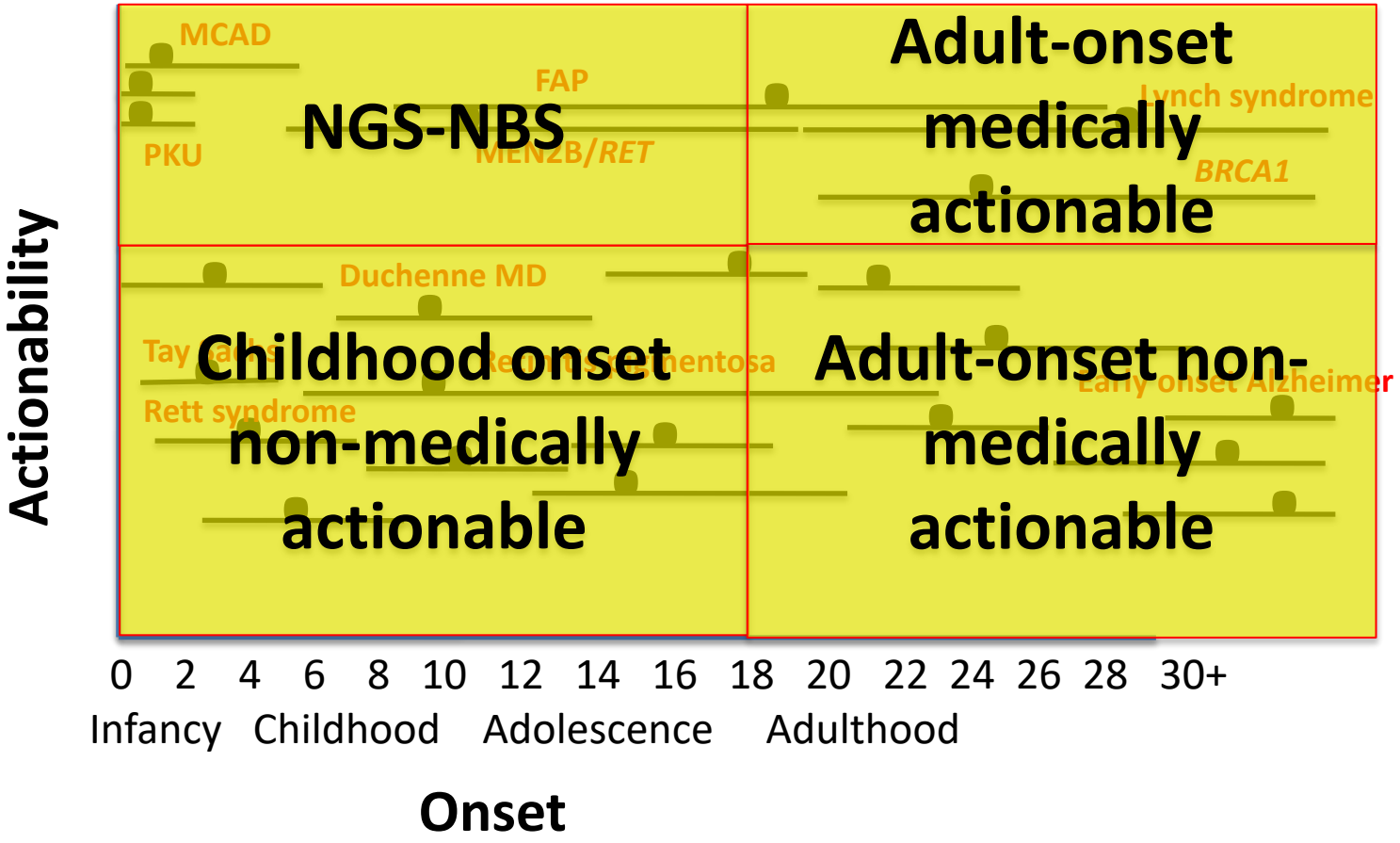
- Severity: possible death due cancer = 2
- Likelihood: high penetrance = 3
- Effectiveness of intervention: colonoscopy = 3
- Acceptability of intervention: colonoscopy = 2
- Knowledge base: high = 3

- Total score of 13

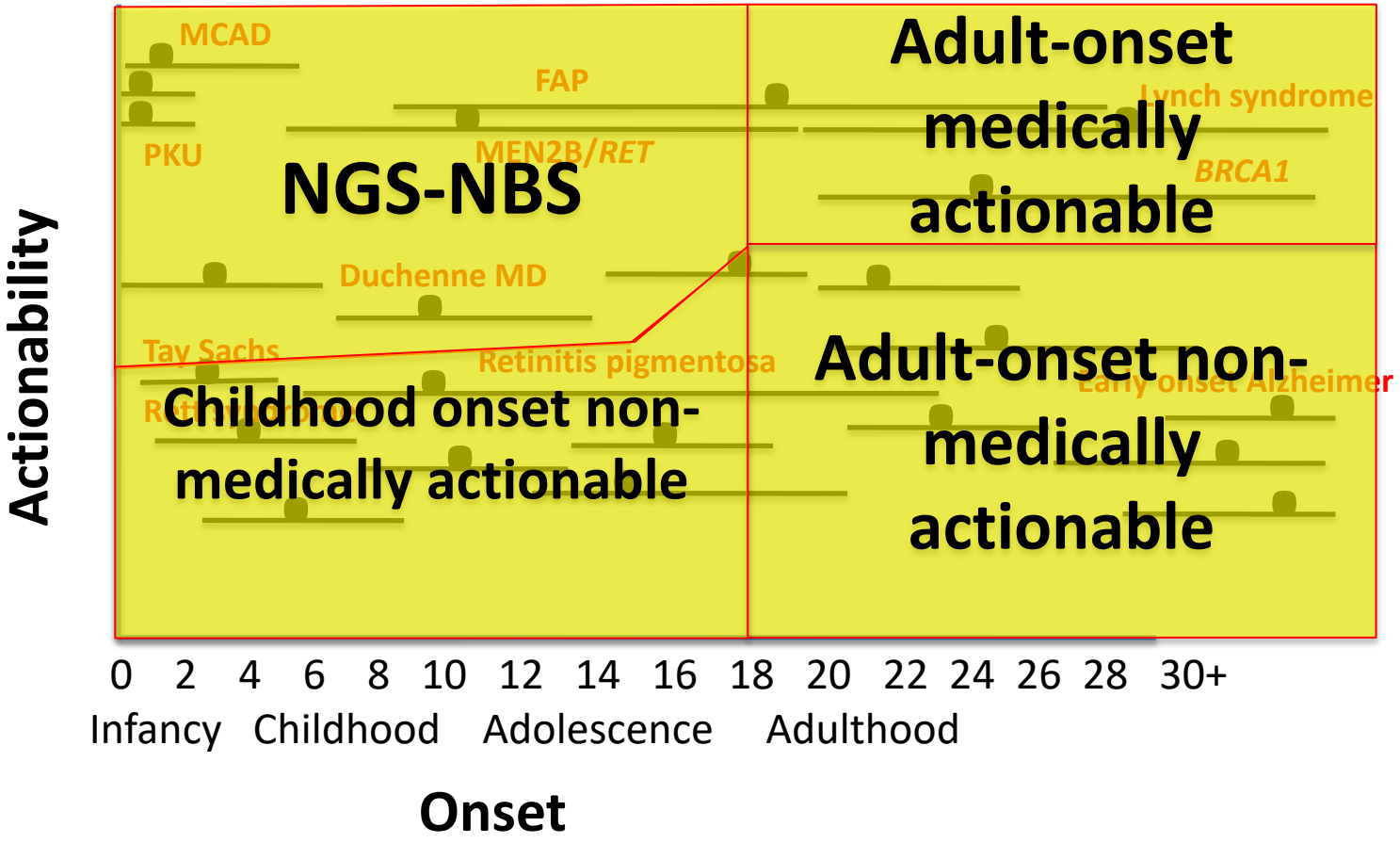
An age-based modified metric system

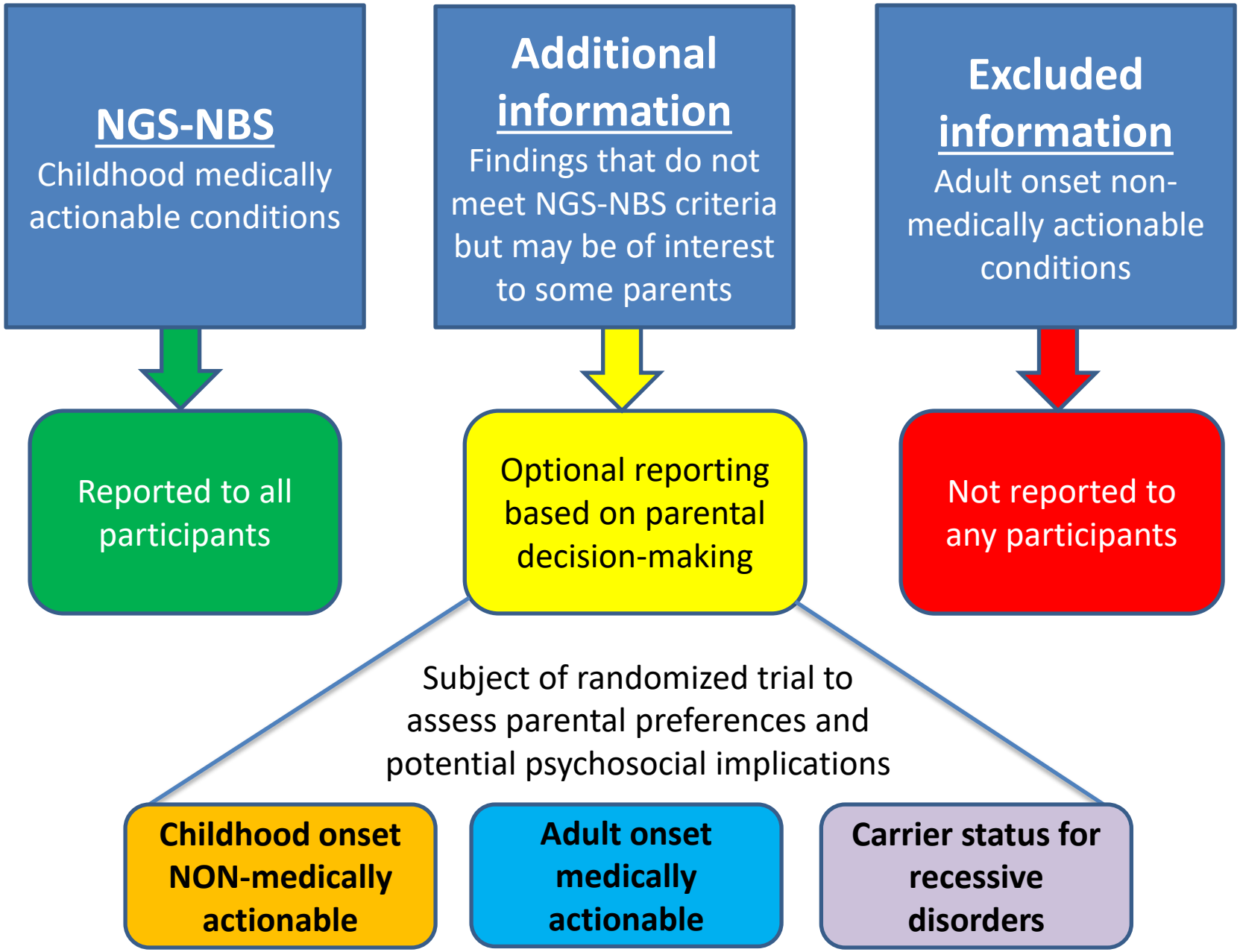


An age-based modified metric system



An age-based modified metric system




NGS-NBS
Childhood medically actionable conditions
Additional information
Findings that do not meet NGS-NBS criteria but may be of interest to some parents
Excluded information
Adult onset non-medically actionable conditions
Reported to all participants
Optional reporting based on parental decision-making
Not reported to any participants
Subject of randomized trial to assess parental preferences and potential psychosocial implications
Childhood onset NON-medically actionable
Adult onset medically actionable
Carrier status for recessive disorders

Informatics – variant calling

- Informatics pipelines convert raw short read data into variant “calls” with associated quality metrics
  - Alignment (currently BWA)
  - Sorting/indexing (currently Picard and Samtools)
  - Variant calling (currently GATK)
- This pipeline is stable, but improvements are expected over time
  - Updated alignment, variant calling algorithms
  - Batch calling as more samples accrue
  - Better identification of indels, other types of variation
- Computational analyses readily allow comparisons to identify optimal algorithms and parameters

Informatics – variant calling

- The primary focus of NC NEXUS is on parental decision-making regarding return of results of whole exome sequencing
  - Not validating a device to be commercialized
  - Most subjects will not have unexpected positive findings
  - Thus, alterations in the informatics pipeline will not affect the main results of the study

Informatics – variant selection

- In NGS, informatics filters play a critical role in selecting which variants will undergo detailed review by a human
  - 100,000 variants per exome
  - 20,000 coding variants
  - 1000's of missense variants
  - 100's of truncating variants
- The vast majority of variants are benign or have uncertain clinical significance
- There is no gold standard method for selecting variants for confirmation in a diagnostic setting
  - Although some groups have started to explore different strategies or establish routine internal practices

Informatics – variant selection

- In the public health setting, human review of variants will be a major limiting factor
  - Because of the rarity of the conditions involved, most samples are expected to have negative results
  - Yet, each individual will have numerous variants in relevant genes (most benign or VUS, others heterozygous and indicating carrier status)
  - Necessitates reliable informatics filters to efficiently filter variant data and identify rare positive results without excessive false positives
- Evaluation of different filtering approaches to identify the optimal parameters is a major research goal of NC NEXUS

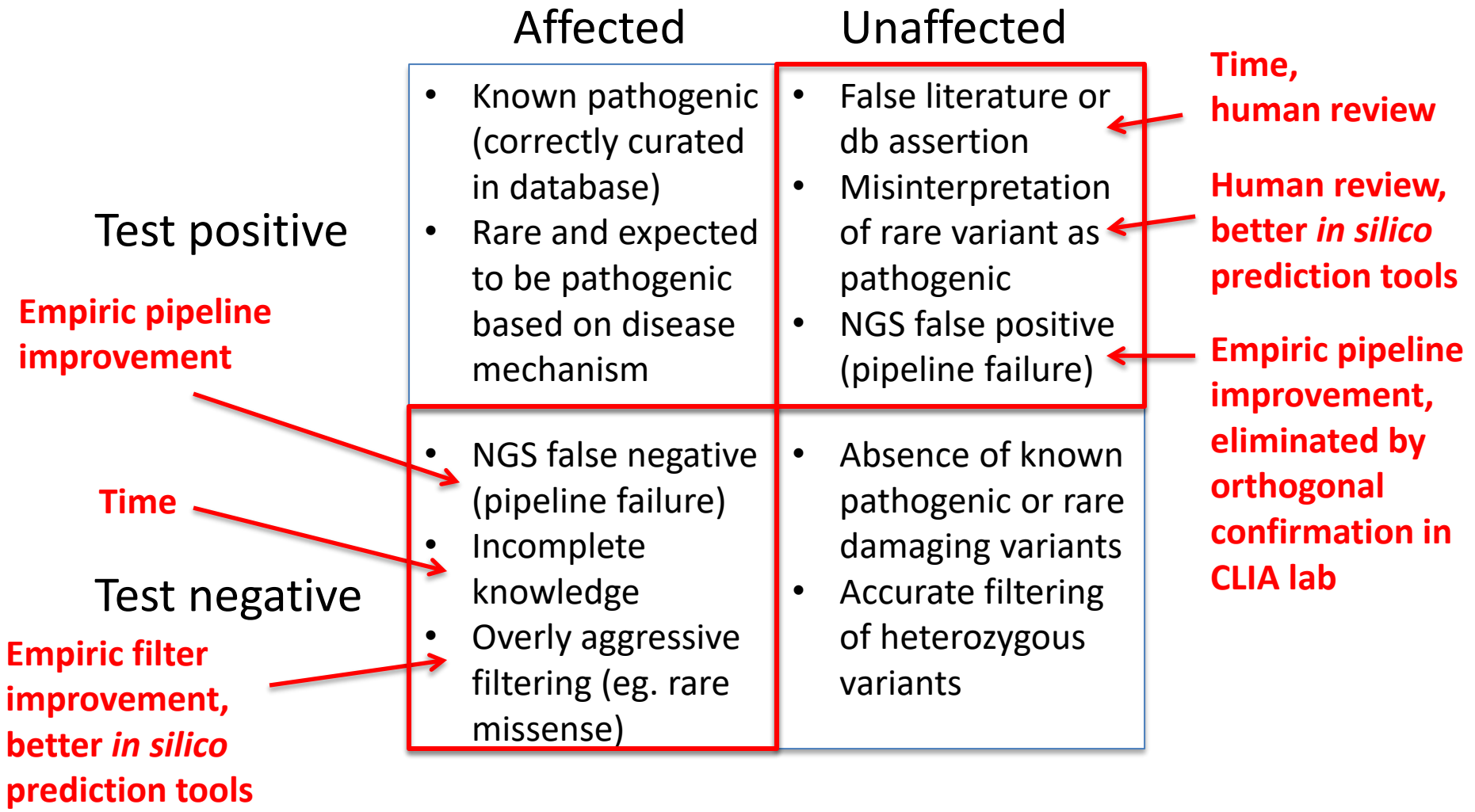
Sensitivity and specificity

	Affected	Unaffected
Test positive	• Known pathogenic (correctly curated in database) • Rare and expected to be pathogenic based on disease mechanism	• False literature or db assertion • Misinterpretation of rare variant as pathogenic • NGS false positive (pipeline failure)
Test negative	• NGS false negative (pipeline failure) • Incomplete knowledge • Overly aggressive filtering (eg. rare missense)	• Absence of known pathogenic or rare damaging variants • Accurate filtering of heterozygous variants

Reasons for false positives

Reasons for false negatives

Sensitivity and specificity



Rationale for modifying informatics

- Optimal parameters for NGS variant calling are not established
- Optimal parameters for variant filtering are not established
- One of the goals of NC NEXUS is to empirically evaluate informatics pipelines for NGS-NBS
- Periodic reanalysis will enhance sensitivity, without sacrificing specificity (due to orthogonal confirmation of any positive findings in the CLIA lab)

What level of risk is involved in the proposed study?

- Were sufficient examples provided for other confirmatory diagnostic methods besides Sanger sequencing?
  - Information about GC
  - Confirmation of orthogonal confirmation

Study design - after internal meeting

What is the risk of returning an unanticipated result – may not impact risk

Clarified how we're determining pathogenic variants

Will our proposed study require an IDE?

- Did we clarify the results that will be returned to parents?
- Did we give adequate information about which results will be placed in the patient's medical record?
- Did we provide sufficient examples of diseases in the distinct binning categories?

What modifications of the protocol are recommended by the FDA?

- If the answer to the previous question is yes, what modifications would be required to alleviate the need for an IDE?

During the course of the study, what changes to the protocol or IRB would require additional review by the FDA?

- Does this response indicate that it will be up to the local IRB to review and determine whether modifications would result in changes to risk?