Clinical Sequencing

Clinical NGS Applications

- Diagnosis of rare genetic diseases
- Cancer precision medicine and treatment selection
- Pharmacogenomics - drug response prediction
- Prenatal and newborn screening
- Infectious disease identification

Clinical Considerations

Quality Standards

- CLIA/CAP certification
- High coverage (>30X)
- Validated pipelines
- Quality control metrics

Interpretation

- ACMG variant classification
- Clinical significance
- Actionable findings
- Secondary findings reporting

Ethical Issues

- Informed consent
- Incidental findings

Reimbursement

- Insurance coverage
- CPT codes

- Data privacy
- Genetic counseling

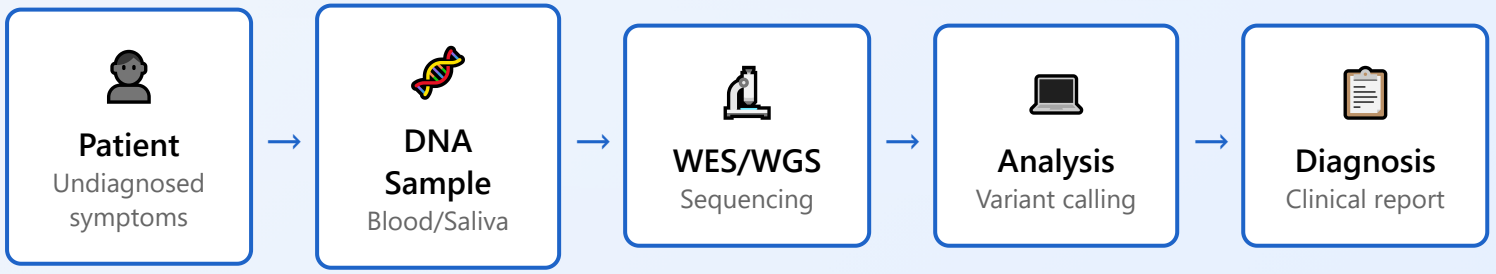
- Medical necessity
- Prior authorization

Requires multidisciplinary team: clinicians, geneticists, bioinformaticians, counselors

Detailed Clinical Applications & Examples

1 Diagnosis of Rare Genetic Diseases

Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) have revolutionized the diagnosis of rare genetic disorders. These technologies enable comprehensive analysis of all protein-coding genes or the entire genome, identifying pathogenic variants that cause disease.



Clinical Case Example

Patient: 6-year-old boy with developmental delay, seizures, and intellectual disability
Previous testing: Karyotype, microarray - negative
WES Result: De novo pathogenic variant in SCN1A gene
Diagnosis: Dravet syndrome
Impact: Changed treatment plan, avoided ineffective/harmful medications, genetic counseling for family

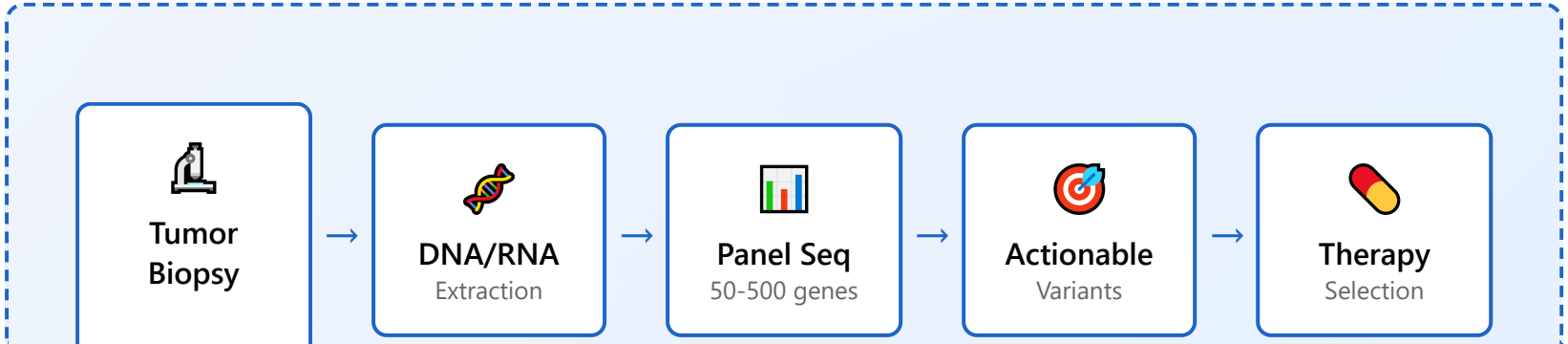
Common Genes Analyzed in Rare Disease Panels

SCN1A | MECP2 | SMN1 | CFTR | DMD | FMR1 | COL4A5 | BRCA1/2

25-50%	~7,000	3-6 weeks
Diagnostic Yield for WES	Known Rare Diseases	Typical TAT

2 Cancer Precision Medicine & Treatment Selection

Tumor sequencing identifies somatic mutations, copy number variations, and gene fusions that drive cancer growth. This information guides targeted therapy selection, predicts treatment response, and monitors disease progression through liquid biopsies.



Tissue/Blood

📌 Clinical Case Example

Patient: 58-year-old woman with stage IV non-small cell lung cancer (NSCLC)
Tumor sequencing: Comprehensive cancer panel (468 genes)
Key findings: EGFR exon 19 deletion, TMB-high (15 mutations/Mb)
Treatment: First-line EGFR TKI (osimertinib) → significant tumor reduction
Monitoring: ctDNA liquid biopsy for resistance mutations (T790M)

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> Actionable Variants Detected:
EGFR: c.2235_2249del15 (p.Leu747_Thr751del) - PATHOGENIC
↳ FDA-approved therapy: Osimertinib, Erlotinib, Gefitinib
↳ Evidence level: 1A (NCCN Guidelines)

PD-L1 expression: 60% TPS
↳ Eligible for pembrolizumab combination therapy

TMB: 15.2 mutations/Mb (TMB-High)
↳ Potential benefit from immunotherapy
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Common Targetable Alterations by Cancer Type

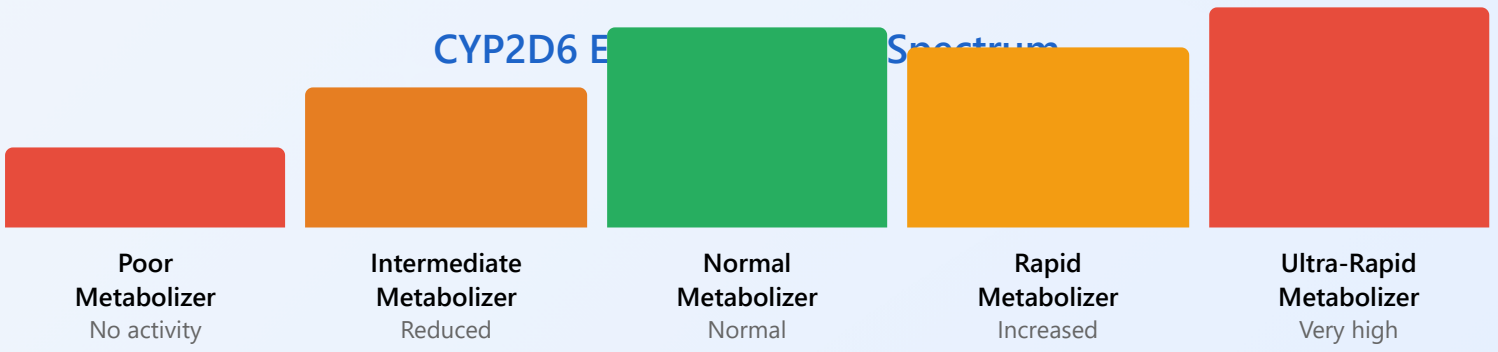
Lung Cancer: EGFR, ALK, ROS1, BRAF, MET, KRAS G12C
Breast Cancer: HER2, PIK3CA, ESR1, BRCA1/2, PALB2
Colorectal: KRAS, NRAS, BRAF V600E, MSI-H, TMB-H
Melanoma: BRAF V600E/K, NRAS, KIT, NF1
Ovarian: BRCA1/2, HRD score, CCNE1 amplification

30-40%	**50-75%**	**7-10 days**
Patients with Actionable Mutations		Typical TAT

Improved Response with Targeted Therapy

3 Pharmacogenomics - Drug Response Prediction

Pharmacogenomic testing analyzes genetic variants in genes encoding drug-metabolizing enzymes, transporters, and drug targets. This enables personalized medication selection and dosing to maximize efficacy and minimize adverse reactions.



📌 Clinical Case Example

Patient: 45-year-old woman starting antidepressant therapy
PGx Testing: CYP2D6, CYP2C19, CYP3A4/5, SLCO1B1
Results:
• CYP2D6: *4/*4 (Poor metabolizer)
• CYP2C19: *1/*17 (Rapid metabolizer)
Interpretation:
• Avoid codeine (no therapeutic effect), tramadol

• Reduce dose of metoprolol by 75%
• Standard dose clopidogrel appropriate

Drug Selection: Venlafaxine selected over paroxetine (CYP2D6 substrate)

Key Pharmacogenes and Associated Drugs

CYP2D6:	Codeine, tramadol, metoprolol, paroxetine, tamoxifen
CYP2C19:	Clopidogrel, omeprazole, escitalopram, voriconazole
CYP2C9:	Warfarin, phenytoin, NSAIDs, losartan
TPMT:	Azathioprine, mercaptopurine, thioguanine
SLCO1B1:	Statins (simvastatin, atorvastatin)
DPYD:	5-fluorouracil, capecitabine (cancer therapy)
VKORC1:	Warfarin dosing
HLA-B*57:01:	Abacavir hypersensitivity

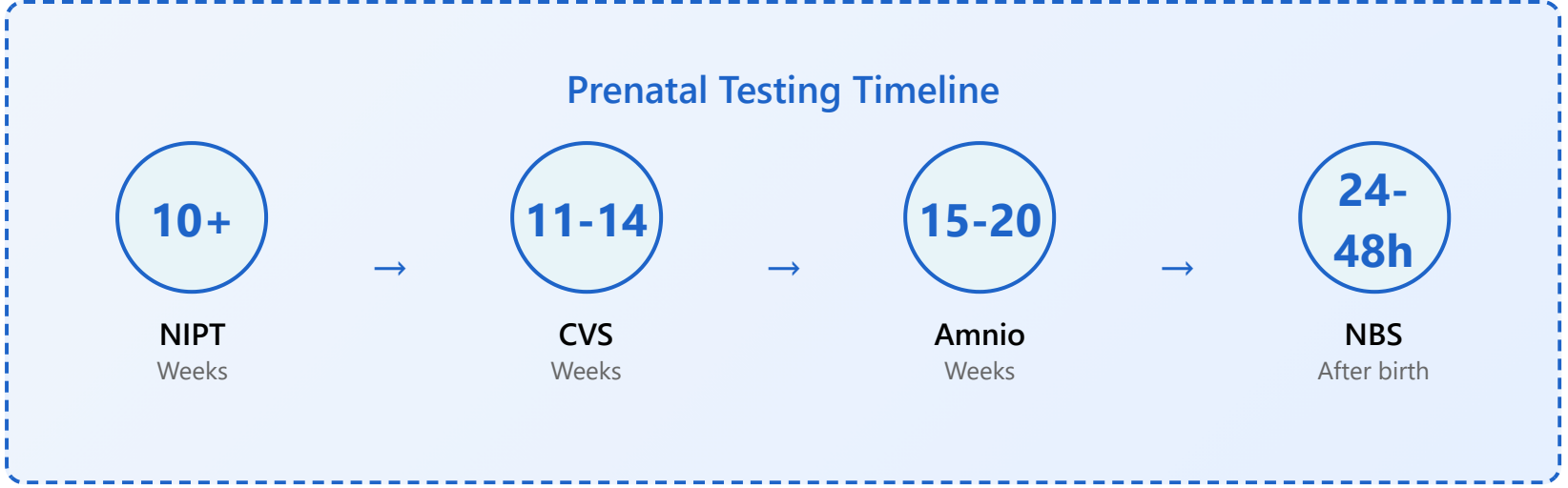
95%
Population with Actionable PGx Variant

30%
ADR Prevention Rate

270+
FDA PGx Drug Labels

4 Prenatal and Newborn Screening

Non-invasive prenatal testing (NIPT) analyzes cell-free fetal DNA in maternal blood to screen for chromosomal abnormalities. Newborn sequencing enables early detection and treatment of genetic disorders before symptoms appear.



📌 Clinical Case Example - NIPT

Patient: 38-year-old pregnant woman, 12 weeks gestation
Test: cfDNA NIPT from maternal blood
Results: Elevated risk for Trisomy 21 (Down syndrome)
Follow-up: Diagnostic amniocentesis confirmed T21
Outcome: Genetic counseling, preparation for specialized care

Detection rates: T21 (99%), T18 (97%), T13 (91%)

📌 Clinical Case Example - NBS

Patient: 48-hour-old newborn
Screen: State mandated newborn screening panel
Positive result: Elevated C8 acylcarnitine
Diagnosis: Medium-chain acyl-CoA dehydrogenase deficiency (MCADD)
Treatment initiated: Avoid fasting, high-carb diet, emergency protocol
Impact: Prevention of potentially fatal metabolic crisis

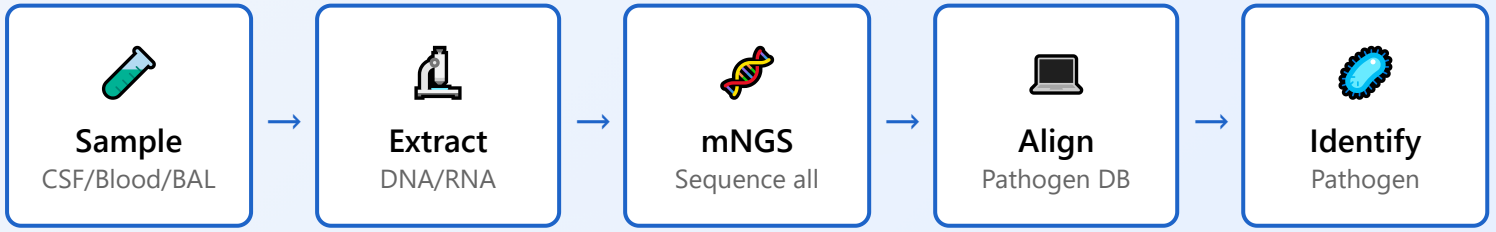
Conditions Screened in Newborns (US Recommended Core Panel)

Organic Acid Disorders: Propionic acidemia, Methylmalonic acidemia, Isovaleric acidemia
Fatty Acid Oxidation: MCADD, VLCADD, LCHAD deficiency
Amino Acid Disorders: PKU, Maple syrup urine disease, Homocystinuria
Hemoglobinopathies: Sickle cell disease, Beta-thalassemia
Endocrine: Congenital hypothyroidism, CAH
Other: Biotinidase deficiency, Galactosemia, Cystic fibrosis, SCID

99%	35+	1 in 300
NIPT Sensitivity for T21	NBS Core Conditions	NBS Detection Rate

5 Infectious Disease Identification

Metagenomic Next-Generation Sequencing (mNGS) enables unbiased detection of all microbial DNA/RNA in clinical samples. This approach identifies pathogens without prior knowledge or culture, crucial for diagnosing unusual or fastidious infections.



Clinical Case Example

Patient: 14-year-old boy with encephalitis, seizures, altered consciousness
Initial testing: Bacterial culture, viral PCR panel - all negative
mNGS (CSF): Performed after 1 week of empiric treatment
Results: *Balamuthia mandrillaris* detected (1,237 reads mapped)
Diagnosis: Rare amoebic encephalitis
Treatment: Switched to appropriate anti-parasitic therapy
TAT: 48 hours from sample to result

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> mNGS Report Summary:
Total reads: 28,456,891
Human reads: 28,442,108 (99.95%)
Non-human reads: 14,783 (0.05%)

PATHOGEN DETECTED:
Organism: Balamuthia mandrillaris
Reads mapped: 1,237
Genome coverage: 12.4%
Confidence: HIGH
Clinical significance: PATHOGENIC - causes GAE
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Clinical Applications of Infectious Disease Sequencing

Meningitis/Encephalitis: Rapid pathogen ID when culture-negative
Sepsis: Blood culture-independent pathogen detection
Pneumonia: Identification of atypical/fastidious organisms
Immunocompromised: Detection of opportunistic infections
Outbreak investigation: Strain typing and transmission tracking
Antimicrobial resistance: Detection of resistance genes
HIV/HCV: Viral load monitoring, resistance mutations
TB: *M. tuberculosis* detection and drug resistance profiling

Advantages over Traditional Methods

✓ Culture-independent (detects non-cultivable organisms)
✓ Unbiased (no prior hypothesis needed)
✓ Rapid results (24-48 hours vs days/weeks for culture)
✓ Detects co-infections

✓ Identifies novel/unexpected pathogens
✓ Simultaneous resistance gene detection
✓ Works with small sample volumes

24-48h
mNGS Turnaround Time

40-70%
Diagnostic Yield in CNS Infections

1000s
Detectable Pathogens

Clinical sequencing has transformed medicine by enabling precision diagnosis and treatment. Each application requires rigorous validation, quality control, and multidisciplinary interpretation to translate genomic data into actionable clinical decisions.