JOHNS HOPKINS
MEDICINE

Challenges for the validation of ctDNA for use in clinical trials

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Disclosure

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"Dr. Gocke is the co-founder and co-owner of OncoMedx. He is also a member of the Board of Directors and a manager of the company. OncoMedx has licensed a number of patents from Penn State University. As an inventor/co-inventor of those patents, Dr. Gocke is entitled to a share of the licensing income. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies."

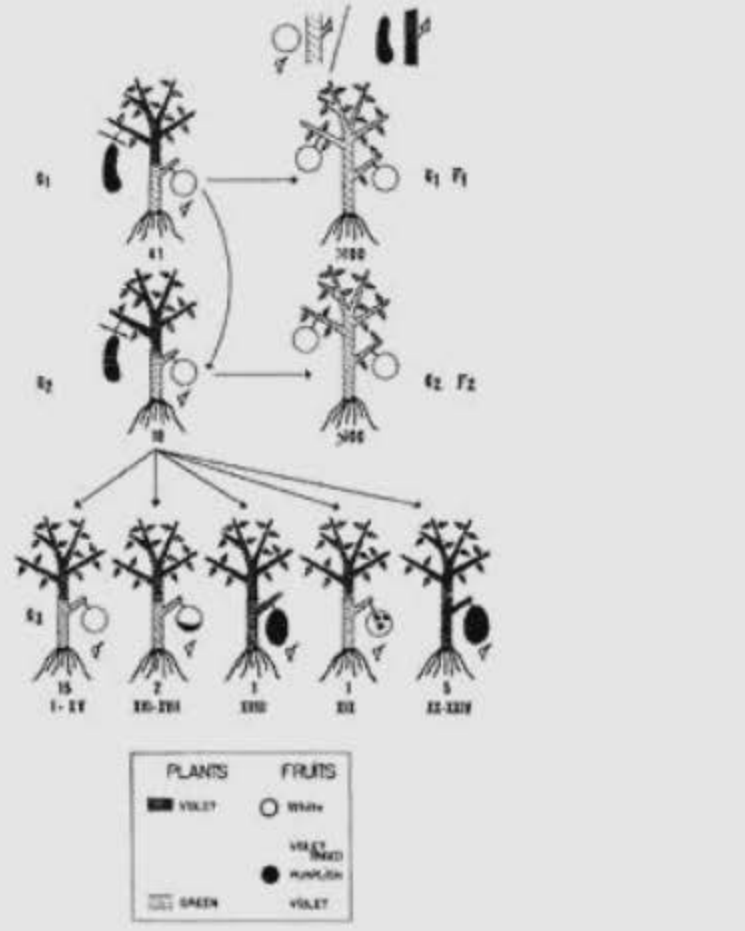
Extracellular DNA in plants

Philippe Anker, Maurice Stroun

ANYAS 1075:10, 2006

Extracellular DNA in plants

- Long purple “teacher” grafted onto round white “pupil”
- After 2 generations, the “pupil” fruit took on the characteristics of the “teacher”



ANYAS 1075:10, 2006

DNA in plasma—early sightings

- 1947, Mandel and Metais (300-1000ng/mL)
- 1960's, rheumatologists in SLE, RA, inflammatory bowel disease
- 1975-85, Leon *et al.*, quantified DNA in plasma of cancer patients and normals (approx.15-fold difference)

Challenges for validation

- Development of standardized protocols
- Purpose-driven test development
  - Screening v. diagnostic v. monitoring
- Analyte selection
- Determine clinical utility for clinical trials

Pre-analytic steps

Research Protocol:

- EDTA or ACD
- First spin: 1,500 x g for 10 minutes
- Second spin: 3,000 x g for 10 minutes
- RT

Commercial protocol #1:

- EDTA
- First spin: 1,900 x g for 10 minutes
- Second spin: 16,000 x g for 10 minutes
- 4 °C

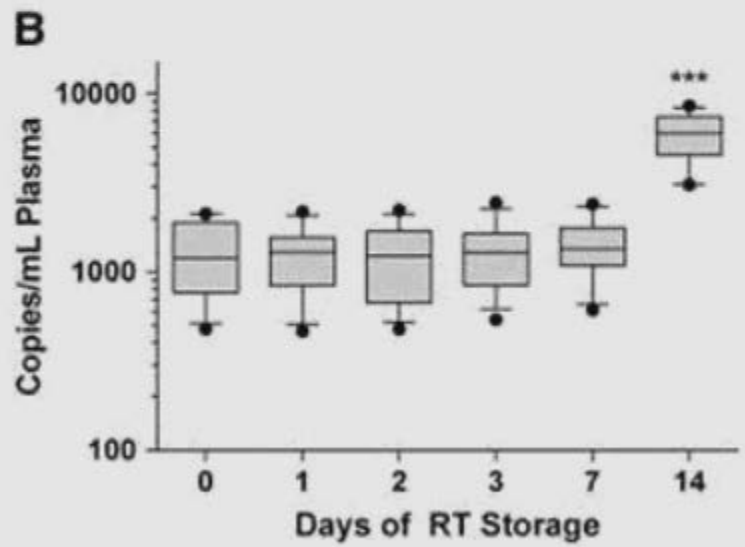
Commercial Protocol #2:

- EDTA
- First spin: 2,000 x g for 10 minutes
- Second spin: 2,000 x g for 10 minutes
- No temp recommended

Pre-analytic steps

- Cell-free DNA BCT tubes (proprietary, Streck)
- Contains fixative
- 2 different centrifugation protocols
- All NIPT companies use DNA BCT tubes

Plasma, twice-spun, n=12
Detection by ddPCR of short amplicons



Clinical Biochemistry (2013) 46:1561

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Cautionary tale—NIPT

- Initially marketed as screen for fetal aneuploidies in high risk pregnancies
- 2015 market estimate: $1.2 billion (1/2 of NGS clinical market)
- 5 US companies offer as LDTs
- >99% sens and spec reported in high risk populations BUT
  - 5% of OB/GYN experts offer NIPT to all patients
  - >50% provide NIPT when asked by the patient
  - 13% offer NIPT as a diagnostic test
- Despite the lack of clinical data on NIPT use for average risk pregnancies and the recommendations of professional societies

Int J Womens Health (2015) 7:113

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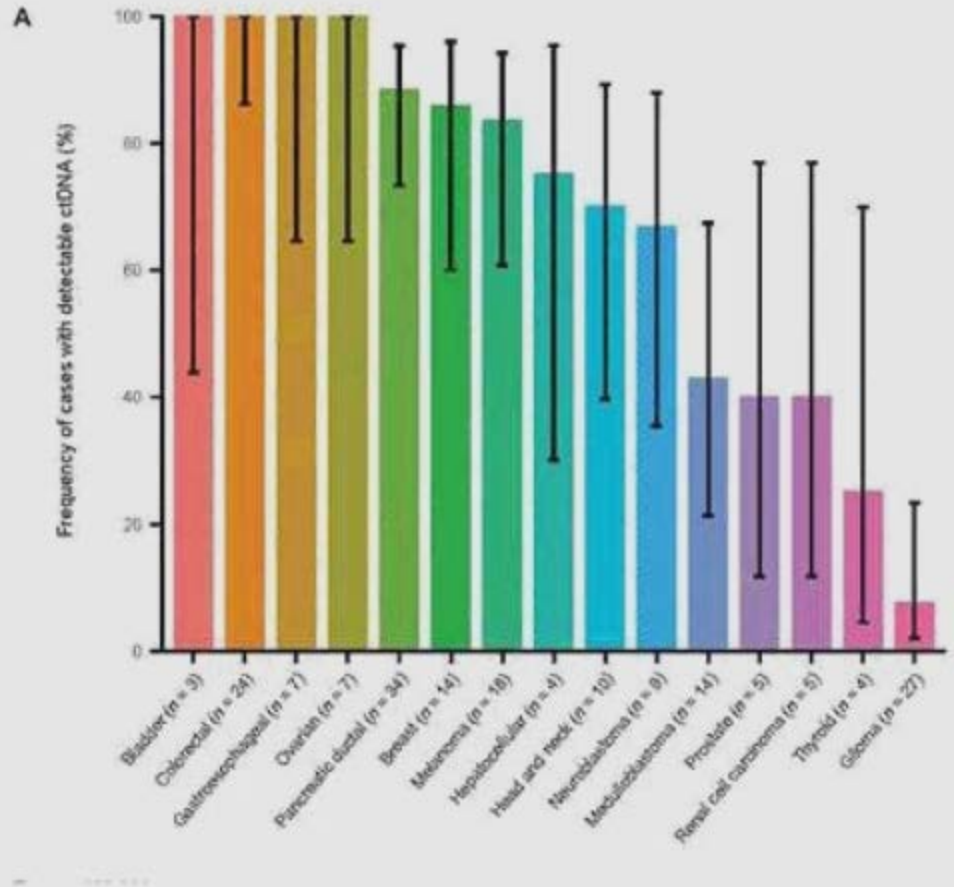
ctDNA is found in most cancers



Bettegowda, Sci Transl Med. (2014) 6:224ra24

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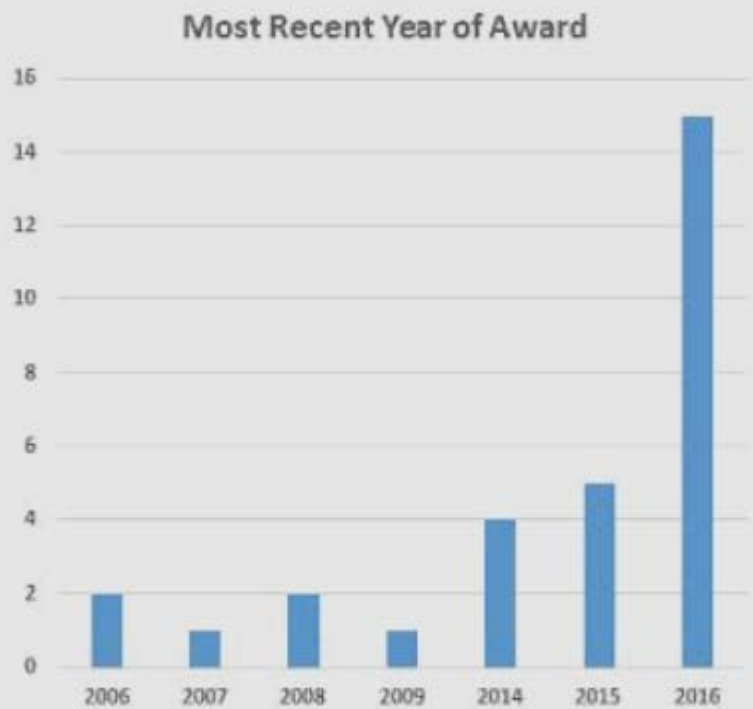
CTEP Checklist for Correlative Studies

- The biologic rationale for studying the proposed target(s) or marker(s)
- The specific hypotheses regarding correlative marker studies (e.g. expression of a marker or markers will define a group of patients whose outcome is so favorable that adjuvant therapy is not required)
- Relevant preclinical data
- Relevant data from previous clinical studies
- The comparability of the methods proposed to those previously used, and the likelihood that the resulting data will be able to be compared with existing data.
- The reason for selection of the assay methodology, particularly in cases where various assays are available that may assess different qualities of the marker; examples: mutation analysis vs. IHC for p53; gene expression vs. protein expression
- The stability of the marker under conditions to be used to collect and preserve the samples.
- The technical performance characteristics of the assay (e.g., reproducibility intra-day and inter-day, accuracy, sources of variability, and how sources of variability will be minimized)
- A description of the positive and negative controls

NIH Support

NIH Reporter Text search: "extracellular DNA" ctDNA "circulating tumor DNA" "circulating nucleic acids"; Excluded cores, intramural

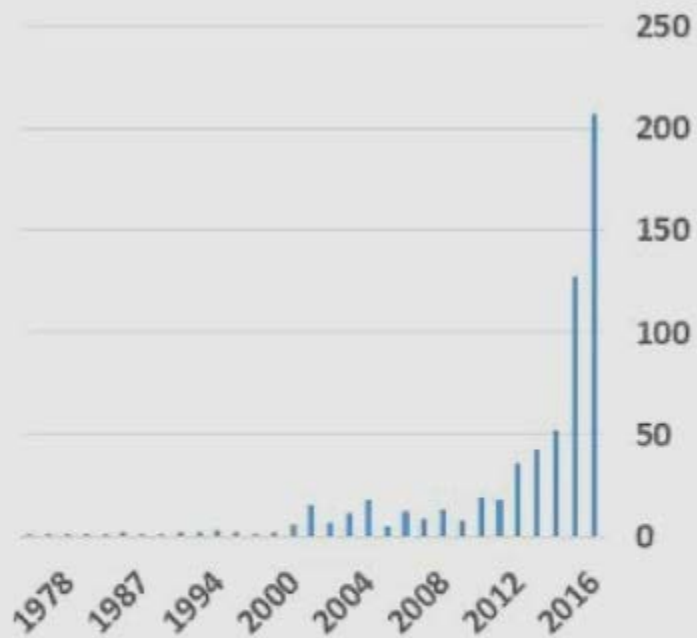


Activity	Count	Total Cost
K99	2	$ 342,096
P50	1	$ 285,911
R01	7	$ 2,633,688
R03	2	$ 153,305
R21	11	$ 1,741,865
R41	1	$ 210,039
R43	1	$ 216,858
U01	4	$ 1,506,303
UH2	1	$ 211,875
	30	**$ 7,301,940**

Pubmed search

Same terms with Cancer OR Tumor

- N=630



Suggestions

- Stop doing proof-of-principle experiments and proceed with large scale validations
- Explore basic biology
  - What is mechanism of ctDNA generation (necrosis, apoptosis, other)?
  - Animal studies (only 3 xenograft papers in Pubmed search)
  - What is quantity of ctDNA generated?
- Determine Clinical Utility
  - Largest study to date: PRESEPT cohort study of *SEPTIN9* methylation as diagnostic of preclinical CRC in standard risk population—1516 people
  - As example of importance of cohort study, prior case/control studies had shown sens of 52-90% (v. 48% PRESEPT) and spec of 88-95% (v. 92%)
  - FDA approved (4/16) *SEPTIN9* methylation test as non-inferior in sens, but not spec, to fecal blood test

ctDNA origin calculations

- "Average" ctDNA quantity ≅ 200-2000 mutant molecules (1.2-12 ng)/mL plasma (Bettegowda)
- 80 kg male has 4000 mL plasma (? interstitial fluid distribution)
- Half life of ctDNA estimated to be 1-2 hours
- Replacement amount of ctDNA is roughly 200 mutants X 4000 mL X ½ X 12 half-lives = 5 to 50 million cells/day
- 1 cm^3 tumor has 10^9 cells (0.5-5%)

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Suggestions

- FoundationACT analytic validation
  - 267 cancer patient samples
  - 117 mixtures from cell lines
  - 42 synthetic DNA samples engineered for complexity
- Clinical validation
  - 2000 person trial on paired tumor/plasma samples to determine concordance