Technology Developments for Liquid Biopsy of Cancer: improving signal to noise ratios for mutation detection

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BWH-DFCI PHYSICS R&D SUMMARY

CLINICAL RADIATION THERAPY
- Motion mgmt & setup
- Image-guided XRT
- Image-guided Brachytherapy

BIG DATA STUDIES
- Radiomics/radio-genomics
- Clinical data mining

PRECLINICAL BIO-PHYSICS
- Nano-radiotherapy
- Blood-based biomarkers
- Nano-particles as radio-sensitizers

BWH-DFCI PHYSICS YEARLY RESEARCH FUNDING (DIRECT $$)

- Image-guided radiotherapy
- Radiomics
- Biophysics

DIRECT FUNDS $$


2017 direct funds $3.1M

External competitive only

JCRT, Mazonne, BWH Kayes awards NEU joint award

calendar (year)
circulating DNA & liquid biopsies
nanoparticles as vascular radiosensitizers
nanoparticles & radiation-enhanced immunotherapy
nanoparticles as drug eluters in brachytherapy
gold nanoparticles RBE & dosim.
Diverse Ct DNA applications: Liquid tumor biopsy; Resistance monitoring, early detection, minimal residual disease
LIQUID BIOPSY IN CANCER: RAPIDLY EXPANDING APPLICATIONS

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Adapted from Haber and Velculescu, Cancer Discov. 2014

- Multiple DNA abnormalities
- RNA expression and fusion transcripts
- Protein expression and phosphorylation
- CTC [cell number]
- Exosomes
  - miRNA; RNA; DNA
  - Amplification and deletion
  - Translocation
  - Point mutations
  - Chromosomal abnormalities
  - Circulating tumor DNA [number of mutant molecules] or aberrant methylation molecules

In vitro/in vivo culture
• **pre-natal diagnosis**
  (i.e. detection of small amounts fetal circulating-DNA in maternal blood)

• **infectious diseases**
  (i.e. early detection of resistant strains emerging in a population of drug-responsive strains)

• **early detection of transplant rejection**
Cancer-Associated Mutations

A **mutation** is a change in the normal base pair sequence.

Commonly used to define DNA sequence changes that alter protein function.
**MULTISTEP CARCINOGENESIS IN THE COLON**

Normal epithelium → Hyperproliferative epithelium → Early adenoma → Intermediate adenoma → Late adenoma → Carcinoma → Metastasis

- Loss of APC
- Activation of K-ras
- Loss of 18q
- Loss of TP53
- Other alterations

Adapted from Fearon ER et al. *Cell* 61:759, 1990
Preparing DNA for Analysis

Blood sample

Centrifuge, then extract DNA either from cells or from free circulating DNA

DNA for analysis
Polymerase Chain Reaction (PCR)

denature DNA  Anneal and extend primers  Repeat as necessary  Amplified segments

Sequence to be amplified

Real time PCR
DNA Sequencing

Start

Normal

Start

Mutant (185delAG)

delA

delG
Circulating tumor DNA reveals patient responses to therapy

cT DNA as early biomarker of immuno-therapy for melanoma metastases

Girotti et al, Cancer Discovery 2016: Liquid biopsy in melanoma
Mutation detection in the immediate post-surgical sample identifies early relapse but misses later relapses.
Dynamic mutation tracking is highly accurate in predicting relapse.

Prediction of relapse in early breast cancer
Prediction of relapse in early breast cancer

Lead time over clinical relapse

ER+ve HER2 +ve breast cancer
pathCR breast/nodes
Neoadjuvant chemotherapy and trastuzumab

Median lead time over clinical relapse for all relapses 7.9 months

Chemotherapy  Letrozole  Trastuzumab

TP53 c.G>T824

13.5M lead over clinical relapse
Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer.
Radiotherapy Response

- 75 y/o F w/ Stage I Adenocarcinoma of right lung
- Tumor measured 2.9 x 2.5 cm
- Had KRAS Gly13Arg mutation
- Treated with stereotactic body RT

![Image of CT scan before and after SBRT treatment]

![Graph showing KRAS mutation over time]

Abhi Patel MD, Ph.D, Radiation Oncology, Yale University
Immunotherapy Response

**Patient 1 - Responder**

- Pretreatment (Day -7)
- Day 418

**Patient 2 - Non-responder**

- Pretreatment (Day -7)
- Day 38

Abhi Patel MD, Ph.D, Radiation Oncology, Yale University
Chemotherapy Response

- 84 y/o F w/ rectal cancer metastatic to liver.
- Began first-line FOLFOX and Avastin with excellent response.
- CT scan at 6 months showed improvement in liver metastases.

Abhi Patel MD, Ph.D, Radiation Oncology, Yale University
Tumor-material is detectable in plasma
Challenges in Measuring ctDNA

- Rare mutant copies in wild-type background.
- DNA is highly fragmented.
- Tumor has genetic heterogeneity
To increase sensitivity-specificity in ccfDNA diagnostics, multiple targets need be interrogated.
DETECTING A SINGLE TARGET vs. DETECTING A FINGERPRINT

Cancer cfDNA in Blood Draw?

- ~5,000,000 genomes of cfDNA per 5 L blood
- ~10,000 genomes of cfDNA per Blood Draw (5-10mL)
  - Mutant cfDNA (166 bp)
  - meth/unmeth DNA
  - WT cfDNA (166 bp)

Genomic Locus
#1 #2 ... #n

FOLLOWING A TUMOR FINGERPRINT IS SUPERIOR TO TRACING ONE MARKER
Next Generation Sequencing Technology:
revolutionizing personalized medicine
and tumor biology

-but good enough for detecting low-level mutations
in heterogeneous tumors or plasma??
Sequencing and sample preparation ‘noise’

2% mutation abundance

1% mutation abundance

TP53 Exon 10, 2% REG, 7574003

seq depth = 1629

TP53 Exon 10, 1% REG, 7574003

seq depth = 1300

NUCLEOTIDE POSITION

ILLUMINA HISEQ SEQUENCING
from Milbury et al, Clin Chem 2012
OUR SOLUTION:
ENRICHMENT OF MUTATIONS
prior to sequencing
1. enrichment during PCR:

2. enrichment for large fingerprint
   NAME – PrO - Song et al, NAR 2016 (mutation)
   - Liu et al, NAR 2017 In Press (methylation)
Nuclease-assisted Mutation Enrichment using Probe-Overlap
Song et al, Nucleic Acid Res 2016

NaME-PrO

Patent pending (DFCI)
NIH R33 2017-2020
DUPLEX-SPECIFIC NUCLEASE, DSN
(Shagin et al, Genome Res 2002)

- Extracted from crab hepatopancreas
- Thermostable - optimal ~ 65°C
- Digests double stranded DNA with high preference over single stranded DNA
- Base mismatches inhibit enzyme
- Previously used for real-time SNP detection; and gDNA ‘normalization’
**Nuclease-assisted Mutation Enrichment using Probe Overlap (NaME-PrO)**

Double strand specific nuclease (DSN, optimal Tm ~ 65°C)

TARGET REGION

BOTTOM STRAND PROBES

TOP STRAND PROBES

Partially overlapping probes
NaME-PrO

Wild type sequence  Mutant sequence

4°C

95°C

lower temperature, add oligos, add DSN

cut  no cut

65°C

PCR

Song et al, NAR 2016
Single-target NaME-PrO on KRAS exon 2

**KRAS** → NaME-PrO → PCR

mutation enrichment → ddPCR

**Wild type KRAS**

**Mutant KRAS**

** KRAS, p.G12V, c.35G>T **

original mutation abundance ~0.5%

![Mutation Abundance (%)](image-url)
Technical detection limit for NaME-PrO-Sanger: IDH1 mutation

NaME-PrO followed by PCR and Sanger Sequencing

1 mutant IDH1 in 3 million WT is detectable
DETECTION OF TUMOR FINGERPRINT IN cfDNA NaME-PrO applied to 54 targets detected in tumor via exome sequencing

- 19 targets (38%) mutation not detected
- 31 targets (62%) mutation detected & enriched

Rare mutation enriched via NaME-PrO and detected in cfDNA with just 15 reads

'noise' limit

DNA TARGETS EXAMINED VIA NaME-PrO-Miseq

No-treat | NaME-PrO
---|---

31 targets (62%) mutation detected & enriched

19 targets (38%) mutation not detected

Rare mutation enriched via NaME-PrO and detected in cfDNA with just 15 reads

'noise' limit
cfDNA from patient 301: serial dilutions into WT cfDNA
13-target tumor fingerprint

ND-NaME-PrO allows extremely sensitive detection of mutation
Large fingerprint cancer screening in blood

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Funded by Bridge Foundation 2017-18
The Problem  
Who needs additional treatment after initial therapy?

Identifying pts with microscopic metastatic disease would enable:
1. Reduction of over treatment of early-stage breast cancer
2. Delivery of new therapies to patients who need them most
3. Determination of whether we can cure patients with metastatic, minimal residual disease
Clinical Design

IF recurrence: cfDNA detected?
IF remission: cfDNA absent?

pathCR

Chemotx

Stage II-III Breast Cancer

Residual disease

cfDNA fingerprint

cfDNA fingerprint

more therapy

pathCR = FDA surrogate endpoint
There is not a single cancer molecule circulating in your body.
From Lo et al, Cancer Res. 2000, 60, 2351-55, ‘Kinetics of Epstein-Barr virus during radiation therapy of nasopharyngeal carcinoma’. EBV levels in plasma, reflecting circulating DNA from tumor cells, peak in the first few days following initiation of radiation therapy, and subsequently decline to undetectable levels.
RADIATION INDUCED LIQUID BIOPSY

Tumor shrinkage during lung SBRT. A centrally located lung tumor treated with 5x10Gy doses at Dana Farber Cancer Institute is displayed on CT. The observed tumor shrinkage corresponds to 2.1 cc tumor tissue releasing genetic material in circulation over 8 days treatment.

COULD RADIATION SERVE AS A NON-INVASIVE BIOPSY TOOL?
Addition of a high dose to a small area of an irradiated lung tumor

Figure 4 Effect of irradiation with thin, intense narrow beams directed at a tumor target area for liquid biopsy purposes: a simulation study. Dose volume histograms (DVH) to nearby Organs At risk (OAR) are calculated before or after adding a 20Gy Liquid Biopsy Boost in 6 fractions (total 120 Gy), during radiation treatment of the right lung with 68Gy standard fractionation. The DVH comparison shows almost overlapping distributions, indicating that irradiation with narrow beams has minimal impact on OARs.

COULD RADIATION SERVE AS A NON-INVASIVE BIOPSY TOOL?
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Funding: NIH / NCI R33 CA217652; and CA180389
Bridge Foundation (DFHCC)