CfDNA in the archived low-quality, low-volume serum samples: rate of concordance with mutations in tumor

International Agency for Research on Cancer
Lyon, France

Dariush Nasrollahzadeh
Behnoush Abedi-Ardekani

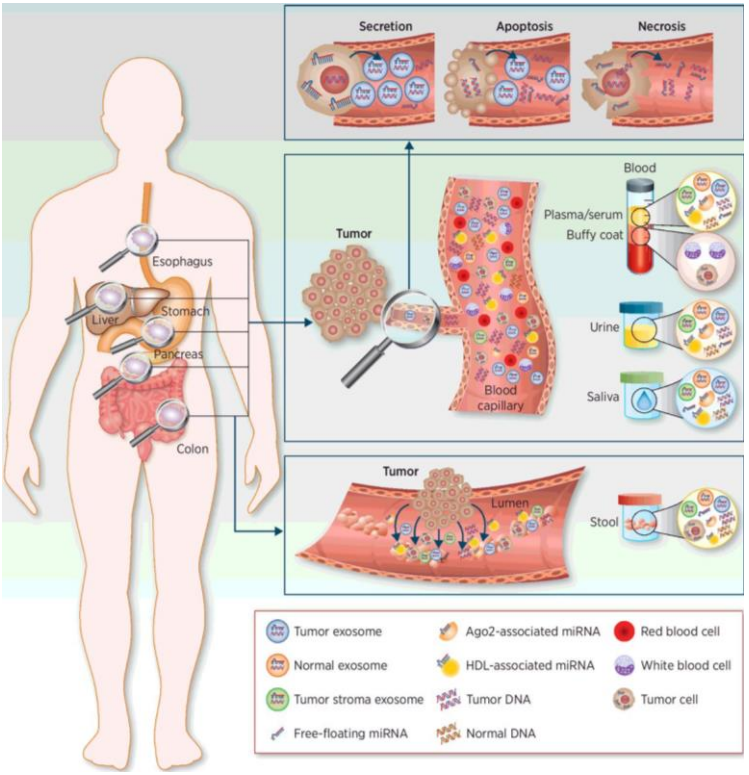
ECP, 9 Sep. 2019
Nice, France

International Agency for Research on Cancer
World Health Organization

return
shift
Conflict of Interest
YES
NO

Concept of liquid biopsy

- Non-invasive approach
- Potential replacement of tissue
- In oncology:
  - Broad range of the malignancy's properties
  - Reflecting the intra-tumor heterogeneity



Background data

Gut. 2010 Sep;59(9):1178-83. doi: 10.1136/gut.2010.210609. Epub 2010 Jun 28.

Polycyclic aromatic hydrocarbon exposure in oesophageal tissue and risk of oesophageal squamous cell carcinoma in north-eastern Iran.

Abedi-Ardekani B[1], Kamangar F, Hewitt SM, Hainaut P, Sotoudeh M, Abnet CC, Taylor PR, Boffetta P, Malekzadeh R, Dawsey SM.

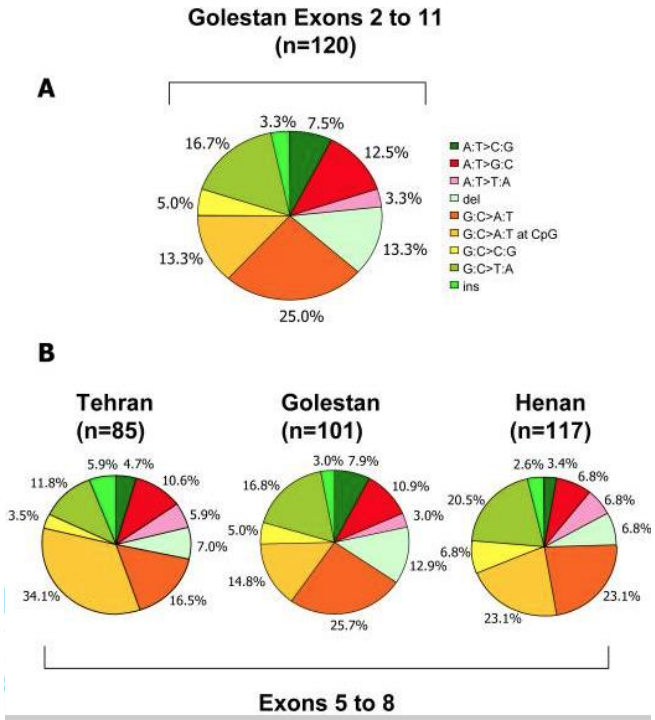
Author information

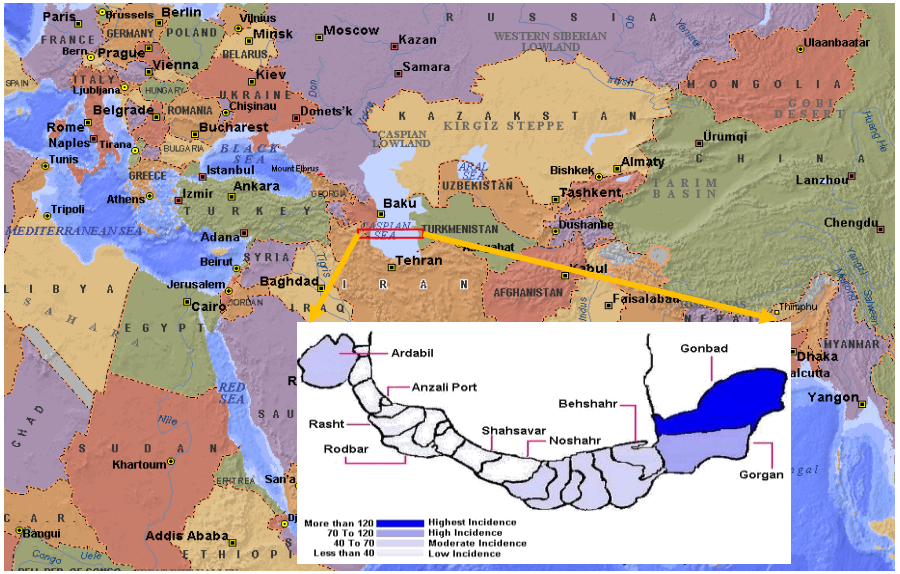
PLoS One. 2011;6(12):e29488. doi: 10.1371/journal.pone.0029488. Epub 2011 Dec 27.

Extremely high Tp53 mutation load in esophageal squamous cell carcinoma in Golestan Province, Iran.

Abedi-Ardekani B[1], Kamangar F, Sotoudeh M, Villar S, Islami F, Aghcheli K, Nasrollahzadeh D, Taghavi N, Dawsey SM, Abnet CC, Hewitt SM, Fahimi S, Saidi F, Brennan P, Boffetta P, Malekzadeh R, Hainaut P.

Author information





ASR=70 of ESCC in the Eastern part of the Golestan Province

- TP53 Mutation rate of 90%
- The most common mutation type: G:C to A:T transitions (38.3%)
- G:C to T:A transversions as the second common type (16.7%)

Main Study

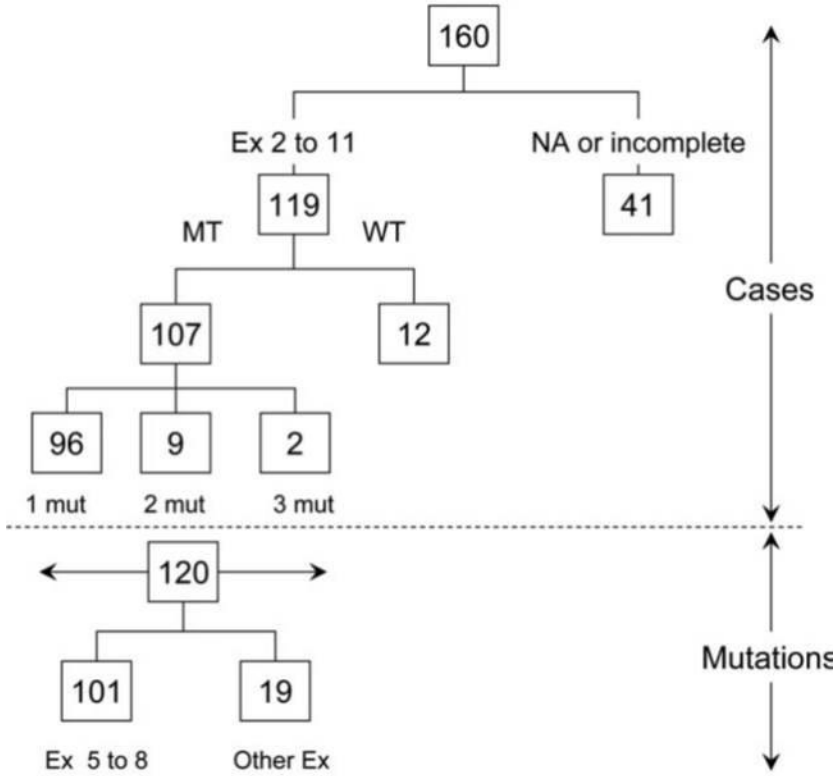
- Identification of tumor mutations in ESCC cases occurring during follow up of Golestan Cohort
- Searching the same mutations in CfDNA in plasma at the time of diagnosis of malignancy
- Searching the same mutations in CfDNA in plasma at the time of recruitment in cohort

Proof-of-Principle

PLoS One. 2011;6(12):e29488. doi: 10.1371/journal.pone.0029488. Epub 2011 Dec 27.

Extremely high Tp53 mutation load in esophageal squamous cell carcinoma in Golestan Province, Iran.

Abedi-Ardekani B[1], Kamangar F, Sotoudeh M, Villar S, Islami F, Aghcheli K, Nasrollahzadeh D, Taghavi N, Dawsey SM, Abnet CC, Hewitt SM, Fahimi S, Saidi F, Brennan P, Boffetta P, Malekzadeh R, Hainaut P.



- To examine if known *TP53* mutations in the ESCC cases can be detected in cfDNA from the serum of the same patients
-

Comparison of pre-analytical recommendations and our archived samples

Recommended	Status of our study samples
Plasma – 1 ml	Serum <0.8 ml (mean 0.5 ml)
Single freeze-thaw cycle	Multiple freeze-thaw cycles >3
Proceed within 6-hours after phlebotomy	Varied
Storage at -80	A year of storage at -20

VOLUME 36 · NUMBER 16 · JUNE 1, 2018
JOURNAL OF CLINICAL ONCOLOGY | ASCO SPECIAL ARTICLE

Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review

Jason D. Merker, Geoffrey R. Oxnard, Carolyn Compton, Maximilian Diehn, Patricia Hurley, Alexander J. Lazar, Neal Lindeman, Christina M. Lockwood, Alex J. Rai, Richard L. Schilsky, Apostolia M. Tsimberidou, Patricia Vasalos, Brooke L. Billman, Thomas K. Oliver, Suanna S. Bruinooge, Daniel F. Hayes, and Nicholas C. Turner

CfDNA concentration of 2.1 to 2.2 ng/ul

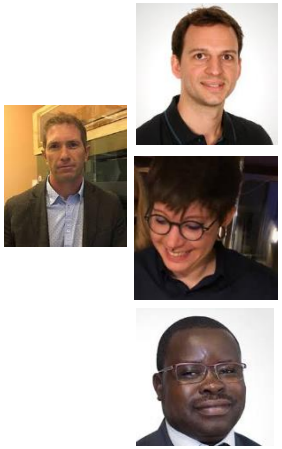
Study design

- Unavailable data on the allelic fraction of tissue mutations
- In-silico selection : positions with low error in sequencing
    - 40 ESCC cases
    - 39 matched controls (age, gender, and residence)
        - To help improving pipeline's calculation of estimates

Sequencing method

- 27 primers covering *TP53* exons and splicing sites were designed and pooled.
- Each sample in duplicate
- Modified GeneRead to amplify *TP53* coding areas
- One pool instead of 4 pools (due to low cfDNA level)
- Libraries at the size of 200-300 bp, Ion-Torrent platform

Needlestack pipeline- variant caller



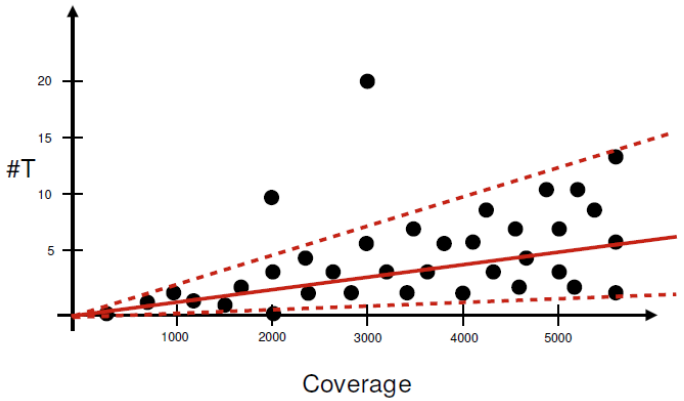
needlestack

A multi-sample somatic variant caller

- For each position/alteration, regression line of coverage and number of alternative determine outliers

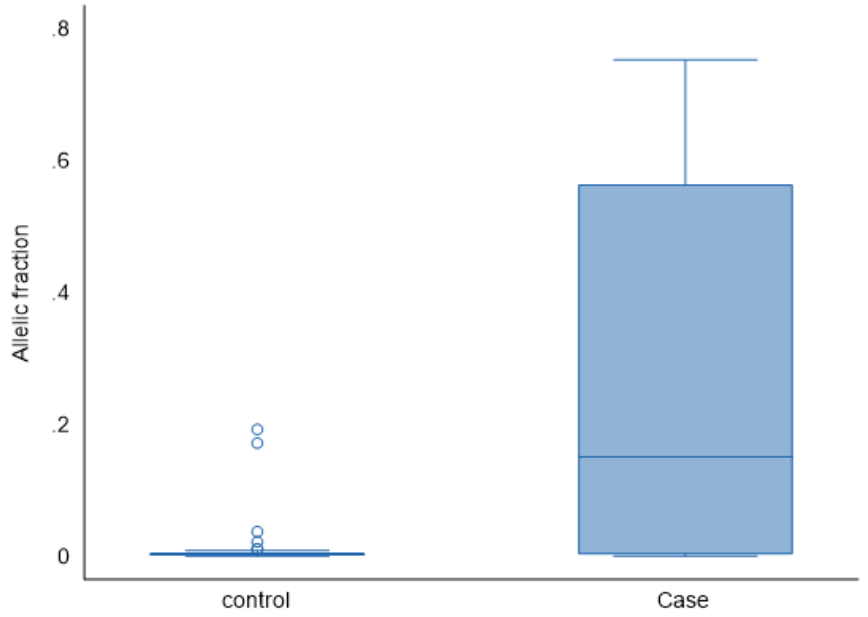
https://github.com/IARCbioinfo/needlestack

- Duplicates to distinguish between sequencing errors and real variants



All mutations

CtDNA Status	Case	Control
cfDNA mutation	23/40	25/39
No of mutations	49	55
Putative effect scoring	1.47	1.23



Comparison with other studies

Published in	Year	Tumor type	Sample type	Sample volume (ml)	mutation in *TP53* cfDNA / tumor (%)
Science	2018	ESCC	Plasma	7 – 7.5	9/40 (22.5%)
Oncotarget	2017	HNSCC	Plasma	0.6 - 2.1	13/45 (29%)
Current study	------	ESCC	Serum	0.5 - 1	9/50 (20%)

*Using duplicate filters will decrease number of reads from 8390 libraries to 220.

Conclusion and future plan

- *Low-volume*, *low-quality* archived *serum* samples can be used for CfDNA extraction and mutation detection
- Our laboratory method in combination of IARC call variant pipeline rendered comparable results to highly cited recent publication
- Combination of different body fluids might improve the mutation detection rate
  - Capsule sponge wash and supernatants
  - Compare with concordant plasma

Acknowledgement

International Agency for Research on Cancer

Dariush Nasrollahzadeh
James McKay
Mathieu Foll
Tiffany Delhomme
Patrice Avogbe
Paul Brennan

Digestive Disease Research Insititute

Reza Malekzadeh, head
Directing upper GI cancer studies in Iran since 2000
All the colleagues in charge of data and
biological material collection

NCI, in 2006 to 2007

Sanford Dawsey
Stephen Hewitt
Christian Abnet
Phil Taylor
Farin Kamangar

Very special thank to Pierre Hainaut,
Former head of the section of mechanism of carcinogenesis at IARC
Current director of Institute of Advanced biotechnology, Grenoble, France
For his mentorship to design and complete the original studies