MATCHED TUMOR NORMAL SEQUENCING:THE ROLE FOR GENETIC COUNSELORS

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OBJECTIVES

- Briefly describe the evolution of cancer genetic testing.
- Define matched tumor normal sequencing.
- Discuss the role of the genetic counselor in matched tumor normal sequencing.

HISTORY OF CANCER GENETICS

- Prior to personalized therapy, cancer treatment was “trial and error”
- Start of personalized therapy → prognostic markers/growth signal inhibitors (factors that predict disease outcome and contribute to the growth and spread of cancer cells; gene-drug interactions)
  - *EGFR, KRAS, BRAF, KIT, Her2,* etc.
- Addition of panels to tumor profiling → no longer looking at specific alleles but at the whole gene
  - More genes = more targets for therapy → potentially better patient outcome

TUMOR PROFILING UTILIZING NGS

- Quickly becoming an integral part of clinical care in oncology
- Utilizes next generation sequencing (NGS) technologies to test for a targeted panel of known cancer-causing genes.
- Identifies acquired (somatic) alterations in the tumor which lead to the malignant phenotype.
- Somatic alterations are targets for personalized therapies and treatments
  - Safer, more effective, and better patient health outcome than traditional treatment

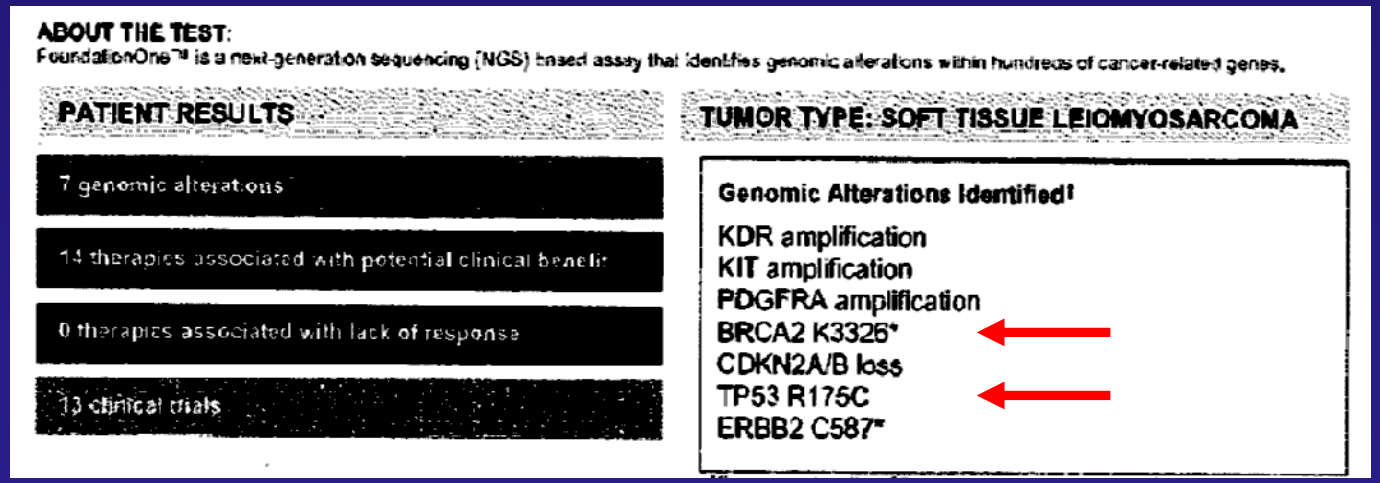
TUMOR GENOTYPING

- Tumor genotyping will identify **BOTH** somatic alterations and germline changes.

- Testing the tumor only cannot always easily distinguish between somatic and germline changes.

CASE EXAMPLE #1

- 62 y/o female
- Breast cancer dx. 44 and leiomyosarcoma dx. 62
- Tumor Profiling



ABOUT THE TEST:
FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

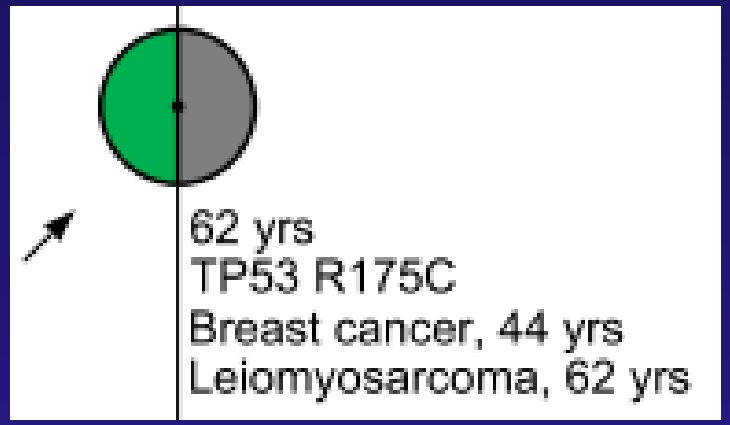
PATIENT RESULTS

7 genomic alterations
14 therapies associated with potential clinical benefit
0 therapies associated with lack of response
13 clinical trials

TUMOR TYPE: SOFT TISSUE LEIOMYOSARCOMA

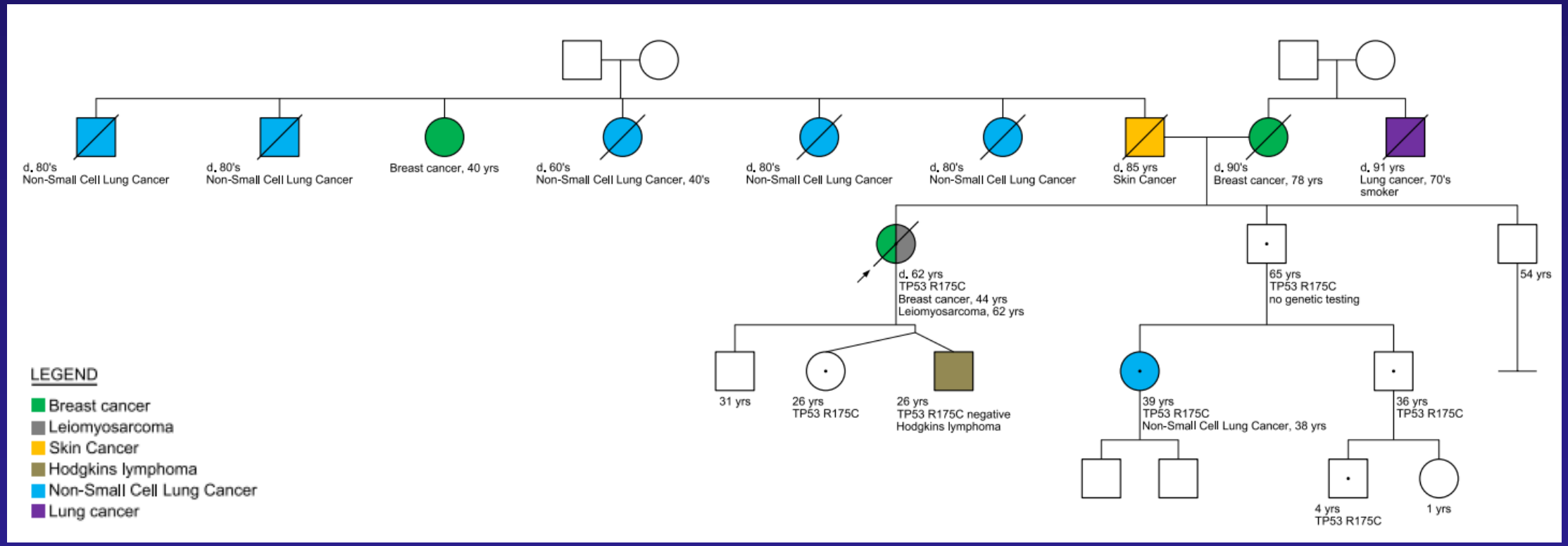
Genomic Alterations Identified†

KDR amplification
KIT amplification
PDGFRA amplification
BRCA2 K3326*
CDKN2A/B loss
TP53 R175C
ERBB2 C587*



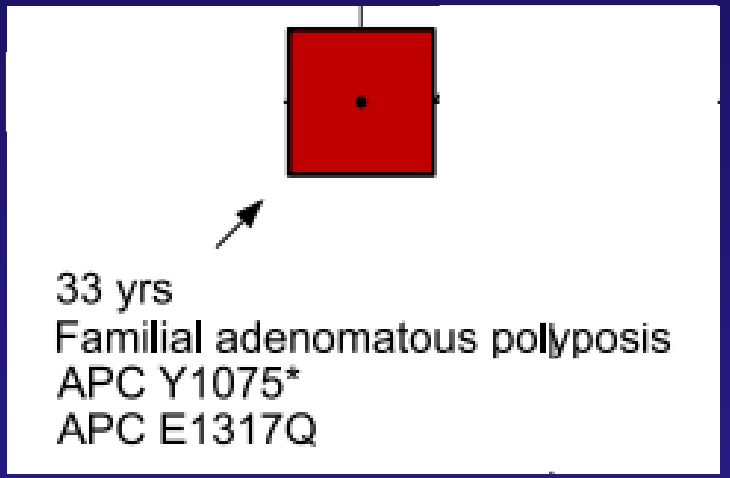
- Referred to Genetics
- MyRisk Panel negative
  - *TP53* R175C VUS

TP53 Family


d. 80's
Non-Small Cell Lung Cancer
d. 80's
Non-Small Cell Lung Cancer
Breast cancer, 40 yrs
d. 60's
Non-Small Cell Lung Cancer, 40's
d. 80's
Non-Small Cell Lung Cancer
d. 80's
Non-Small Cell Lung Cancer
d. 85 yrs
Skin Cancer
d. 90's
Breast cancer, 78 yrs
d. 91 yrs
Lung cancer, 70's
smoker
d. 62 yrs
TP53 R175C
Breast cancer, 44 yrs
Leiomyosarcoma, 62 yrs
65 yrs
TP53 R175C
no genetic testing
54 yrs
31 yrs
26 yrs
TP53 R175C
26 yrs
TP53 R175C negative
Hodgkins lymphoma
39 yrs
TP53 R175C
Non-Small Cell Lung Cancer, 38 yrs
36 yrs
TP53 R175C
4 yrs
TP53 R175C
1 yrs
LEGEND
Breast cancer
Leiomyosarcoma
Skin Cancer
Hodgkins lymphoma
Non-Small Cell Lung Cancer
Lung cancer

CASE EXAMPLE #2

- 33 y/o male
- Colon cancer and polyposis dx. at 31 y/o
- Met clinical criteria of FAP
  - → full gene sequencing *APC*
    - Y1075* and E1317Q
- Tumor Profiling one year later…



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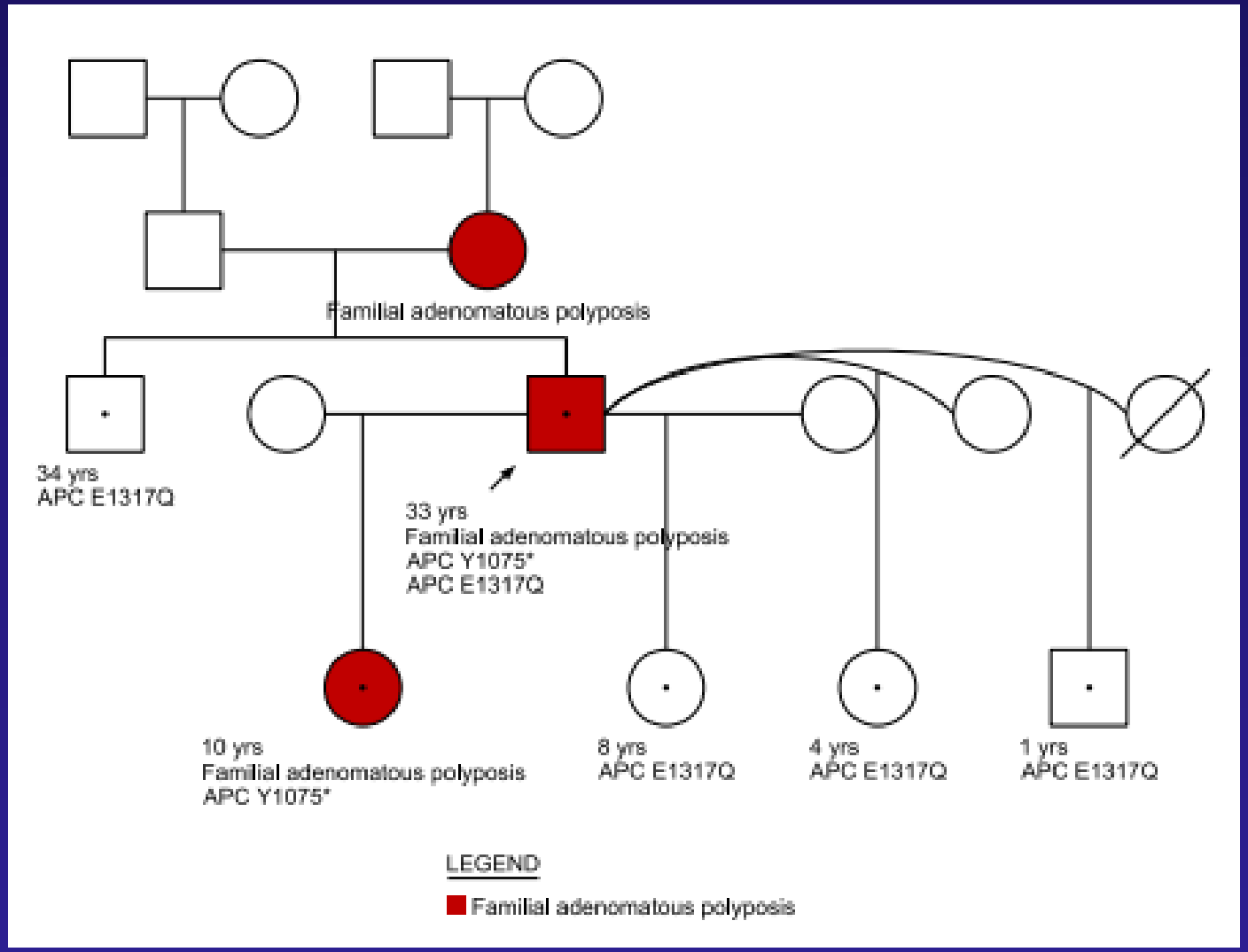
PATIENT RESULTS	TUMOR TYPE: COLON ADENOCARCINOMA (CRC)
5 genomic alterations	**Genomic Alterations Identified†** *KRAS* G12R *FBXW7* R465C *PTEN* loss *APC* E1379*, Y1075* ←
0 therapies associated with potential clinical benefit	
2 therapies associated with lack of response	
14 clinical trials	**Additional Disease-relevant Genes with No Reportable Alterations Identified†** *NRAS* *BRAF*

†For a complete list of the genes assayed and performance specifications, please refer to the Appendix

APC Family


Familial adenomatous polyposis
34 yrs
APC E1317Q
33 yrs
Familial adenomatous polyposis
APC Y1075*
APC E1317Q
10 yrs
Familial adenomatous polyposis
APC Y1075*
8 yrs
APC E1317Q
4 yrs
APC E1317Q
1 yrs
APC E1317Q
LEGEND
Familial adenomatous polyposis

Case Examples

- Both received tumor profiling and germline sequencing
  - Timing different
  - Different stages of in their cancer history
    → essentially matched tumor normal sequencing
- Genetic counseling involvement only during germline sequencing

MATCHED TUMOR-NORMAL SEQUENCING

- Matched tumor-normal sequencing
  - Comparing tumor and matched normal sequencing pairs
  - Allows for correct identification of somatic and germline alterations
- Not widely utilized
  - Due to cost and turn-around-time
- Why is this important?
  - Allows for informed and appropriate treatment decisions
  - Identifies personal and familial clinical implications
    - Could be missed without performing matched tumor-normal sequencing

WHAT HAS BEEN SEEN SO FAR…

- One study indicated that 31% of alterations identified in tumor samples may be incorrectly classified. This leads to pathogenic germline changes being missed or germline variants mistakenly classified as actionable somatic changes. (Jones at al. 2015)
- Another study suggested that 16% of tumor alterations were germline.
  - Almost every participant had more than one germline variant of uncertain significance.
  - (Schrader et al. 2015)
- Indicates a high incidence of tumor alterations are germline rather than somatic alterations.

AN ADDITIONAL MATCHED TUMOR NORMAL STUDY

- My graduate school thesis project (through UAMS)
- Conducted at Sanford Health in Sioux Falls, SD

GEMMA TRIAL

- Genetic Exploration of the Molecular Basis of Malignancy in Adults
  - Oncogenetic clinical trial
  - Individuals with cancer whose disease has progressed following their first line of treatment
    - Purpose – determine a better course of cancer treatment
- All participants received tumor profiling
  - 106 enrolled (as of September 2015)
- Trial already in place – patients already consented to germline testing in original GEMMA protocol
- The purpose of GEMMA was not to identify germline variants but it is a question that comes up

TUMOR PROFILING

PATIENT RESULTS

- 4 genomic alterations
- 0 therapies associated with potential clinical benefit
- 0 therapies associated with lack of response
- 2 clinical trials

TUMOR TYPE: STOMACH NEUROENDOCRINE CARCINOMA

Genomic Alterations Identified†

CCNE1 amplification
TP53 R110_L111insR
KDM6A loss exons 18-29
RB1 loss exons 21-27

APPENDIX

VARIANTS OF UNKNOWN SIGNIFICANCE

Note: One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants have not yet been adequately characterized in the scientific literature. We choose to include them here in the event that they become clinically meaningful in the future.

AKT2 R208K	**ALK** R1231Q	**MAP2K1** P15A	**NTRK3** S716Y
PTCH1 R13G	**ZNF703** H402_D403>PTHLG GSSCSTCSAHD		

FREQUENCY OF ALTERATIONS

- Actionable Variants
  - 146 actionable variants in 57 genes
  - Average number of actionable variants = 4.29 actionable variants per individual
  - Range = 1-12 actionable variants
- VUSs
  - 419 VUSs in 199 genes
  - Average number of VUSs = 12.32 VUSs per individual
  - Range = 3-93 VUSs

GERMLINE SEQUENCING

- Performed at Sanford Health
- Next Generation Sequencing
- TruSightOne focused exome kit from Illumina
  - 4200 genes
  - Ran on HiSeq 2500
- GenomePilot software (Appistry) was utilized to run BWA-MEM and GATK to generate the variant call files (VCF)
- VCF files loaded into the Codified software
  - Only looked at *TP53* and the 314 other genes that were on the Foundation One reports

FREQUENCY OF GERMLINE ALTERATIONS

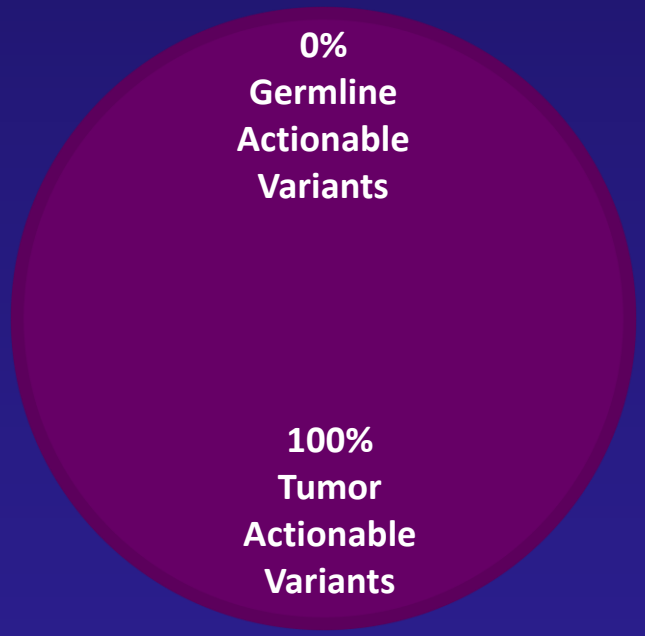
- **None** of the actionable variants identified in the tumor were found in germline.

- VUSs
  - 84 VUSs in 57 genes
  - Average number of VUSs = 2.47 VUSs per individual
  - Range = 0-5 VUSs

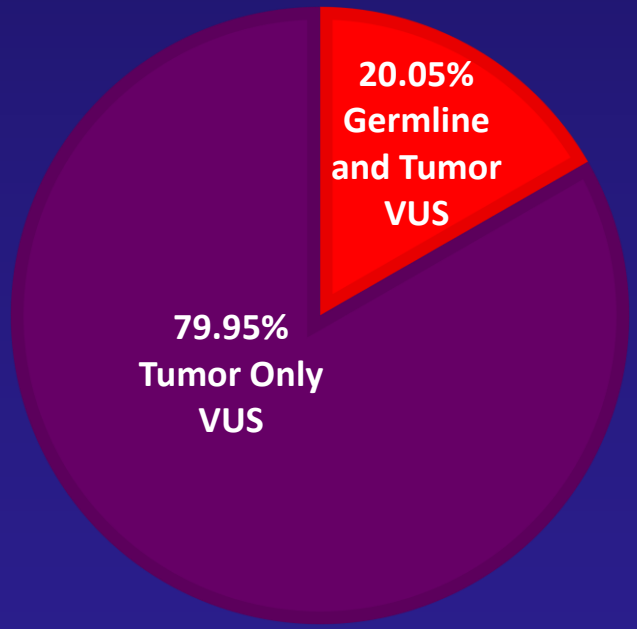
- **31 of 34 (91.18%)** individuals had at least 1 germline VUS

TUMOR REPORTED vs GERMLINE REPORTED - MUTATIONS and VUS

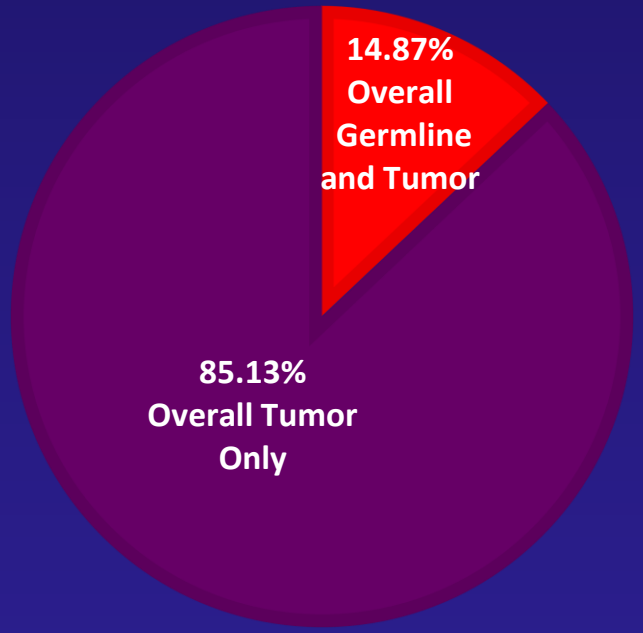
% Germline Actionable Variants



% Germline Variants of Uncertain Significance



% Total Germline Variants



MATCHED TUMOR NORMAL SEQUENCING SUMMARY

- Comparing tumor and matched normal sequencing pairs
- Allows for correct identification of somatic and germline alterations
- Allows for informed and appropriate treatment decisions
- Identifies personal and familial clinical implications
- Previous studies indicate a high proportion of alterations identified on the tumor profile are germline rather than somatic

GENETIC COUNSELING IN MY STUDY

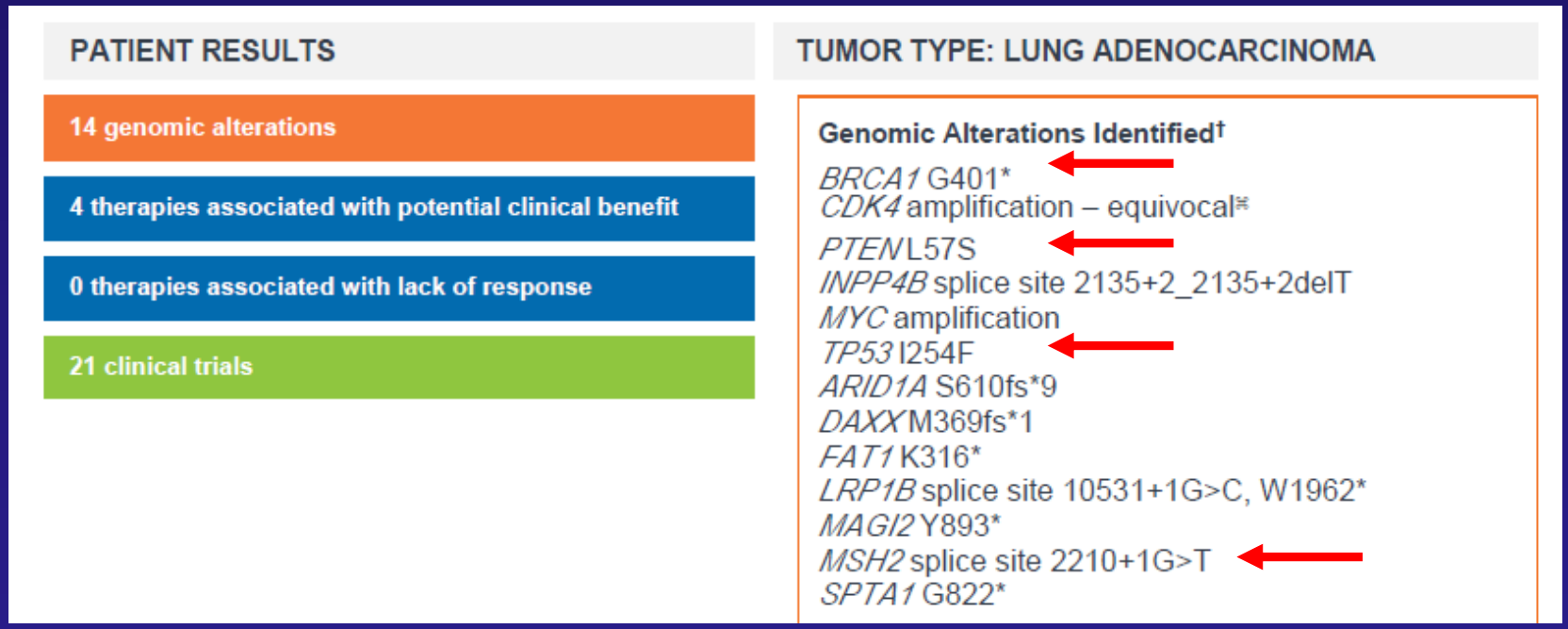
- 4 individuals were referred for genetic counseling
  - 1 passed prior to genetic counseling
  - 3 received genetic counseling and germline testing
    - 1 prior to tumor profiling
    - 2 after tumor profiling

GENETIC COUNSELING EXAMPLE

- Male
- Lung adenocarcinoma – dx. 67 y/o
- Family history
  - Brother – lung cancer dx 59 y/o
  - Sister – breast cancer dx 50s
  - Sister – breast cancer dx 58 y/o
  - Maternal uncle – lung cancer
  - Maternal uncle – esophagus and colon cancer
  - Paternal grandmother – uterine cancer dx 33 y/o
  - Paternal uncle – bone cancer/sinus cancer dx 70s

GENETIC COUNSELING EXAMPLE

- Tumor Profiling:



  - *MSH2 , BRCA1 , PTEN , TP53*
- Referred for germline testing after tumor profiling

GENETIC COUNSELING EXAMPLE

- Germline Testing:
  - *BRCA1, BRCA2, CHEK2, EPCAM, MLH1, MSH2, MSH6, PMS2, MUTYH, PTEN, TP53*
  - 2 VUSs identified
    - *MLH1* c.-28A>G
    - *MLH1* duplication exon 16-19
      - **Neither** were identified in the tumor profile

WHAT ROLE DO GENETIC COUNSELORS PLAY IN MATCHED TUMOR NORMAL SEQUENCING?

- Assess family history regardless of cancer diagnosis
- Follow-up on tumor profiling reports – both actionable variants and variants of uncertain significance
- Facilitate germline sequencing (if appropriate)
- Identify other family members who would benefit from genetic counseling and/or genetic testing
- Provide risk assessment and communication
- Patient education and assure patient understanding

INTEGRATING GENETIC COUNSELING

- Integration into multidisciplinary oncology teams
- Involvement in tumor boards and case conferences
- Involvement in the patient's initial or follow-up oncology visits
- Genetic counselors can help coordinate care
  - Help clinicians determine appropriate follow-up
  - Coordinate familial testing and care
  - Bring together/connect patients and their families that have seen multiple providers (oncology, genetics, etc.)

TAKE HOME POINTS

- Matched tumor-normal sequencing is effective in detecting somatic versus germline variants.
- Establishing tumor-normal profiling as clinical practice will assist in the care and treatment of cancer patients, as well as assess future health risks.
- There should be greater involvement of genetic counselors in these types of cases.
  - Genetic counselors play an important role in risk assessment, patient counseling and education, and case management.

QUESTIONS

http://www.nhs.uk/conditions/vaccinations/pages/parents-questions-about-childhood-vaccinations.aspx

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