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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
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Clinical molecular profiling of archival tumor tissue and cell-free DNA from patients with Erdheim-Chester disease

Filip Janku, Eli Diamond, Vaijayanthi Kandadai Raghavan, Aaron Goodman, Tamara G. Barnes, Helen J. Huang, Shumei Kato, Razelle Kurzrock

Memorial Sloan Kettering Cancer Center
1884

UC San Diego
Moores Cancer Center

Rationale

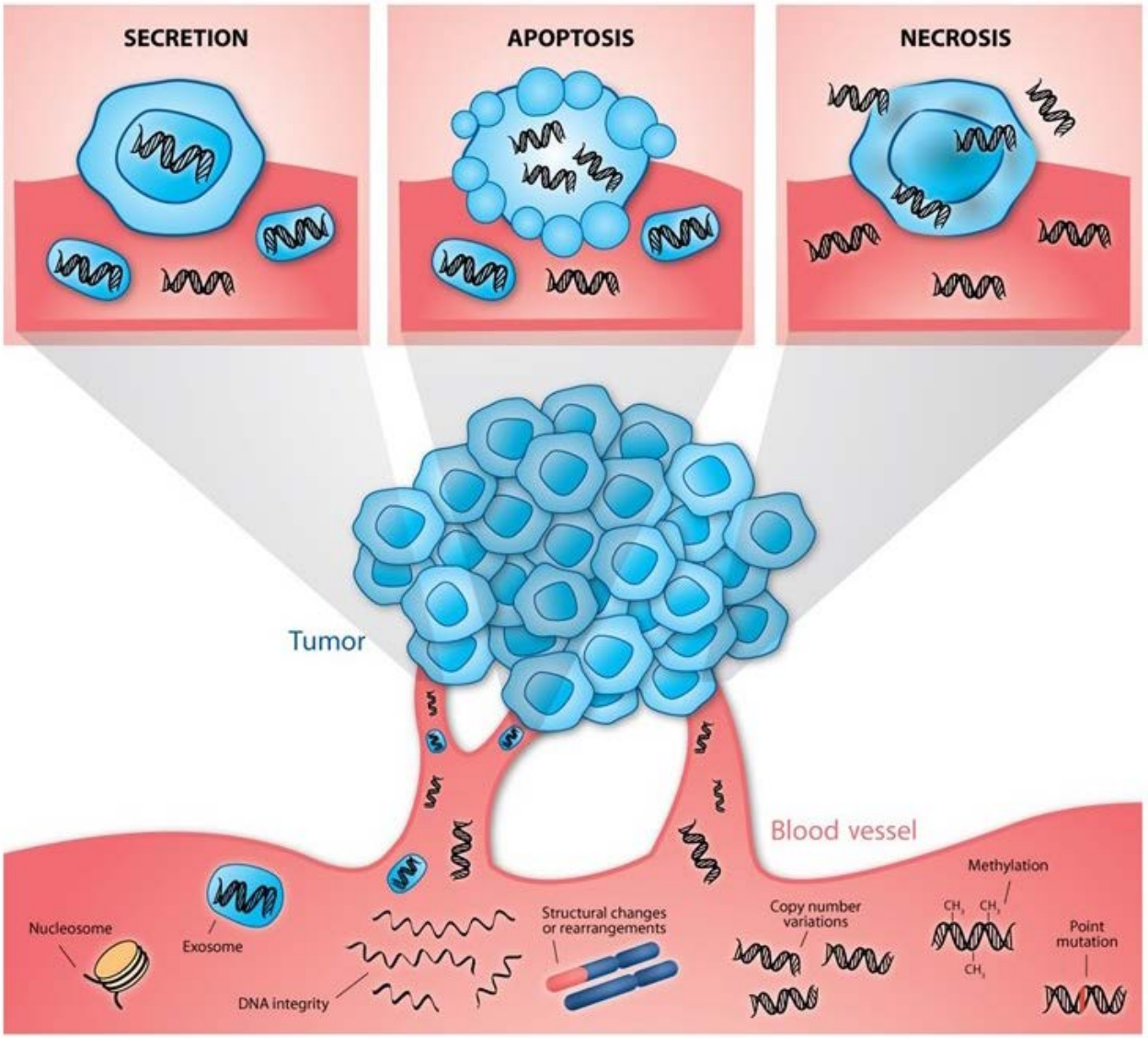
- *BRAF*^{V600E} mutations and other druggable molecular alterations can be detected in majority of patients with Erdheim-Chester disease (ECD)

- ECD patients with *BRAF*^{V600E} mutations and other druggable molecular alterations can respond to appropriately selected targeted therapies (e.g. BRAF and MEK inhibitors)

- Molecular testing of tumor tissue is often problematic in patients with ECD especially in patients with bone disease

Haroche J. Blood 2012
Diamonnd EL. Cancer Discov 2016

Concept of “liquid” biopsy



Polivka, Janku. Expert Rev Mol Diagn 2015

BRAF mutations in Erdheim-Chester disease (non-Langerhans cell histiocytosis) with droplet digital PCR

Patient #	Urine *BRAF* V600E/WT	Plasma *BRAF* V600E/WT	Patient Tissue *BRAF* status
1	V600E (22.59%)	V600E (8.598%)	V600E
2*	V600E (0.311%)	V600E (1.522%)	V600E
3	Wild-type (0.010%)	Wild-type (0.063%)	Wild-type
4	V600E (0.159%)	Wild-type (0.047%)	Unknown**
5	V600E (4.940%)	V600E (0.261%)	Unknown**
6	Wild-type indeterminate (0.079%)	Wild-type (0.048%)	Unknown**

*Urine and plasma collected on different dates

** Insufficient tissue for molecular analysis

Janku et al. Oncotarget 2014

Methods: all CLIA compliant

- Isolation of tumor DNA, plasma and urine cell-free (cf) DNA
- Molecular testing of tumor tissue
  - PCR
  - Targeted NGS (Ion Torrent, Foundation One, IMPACT)
- Molecular testing of plasma cfDNA
  - Targeted NGS (Guardant 360)
- Molecular testing of urine cfDNA
  - PCR (Trovagene)

Patients

- **Total of 38 patients from 3 Care Center (MDA, UCSD, MSKCC)**
  - MDA: 23
  - UCSD: 6
  - MSKCC: 9

- **Type of non-Langerhans histiocytosis**
  - ECD: 36
  - Rosai-Dorfman Disease (RDD): 2

- **Male/Female**: 22/15

- **Ethnicity** – white/black/other: 27/3/8

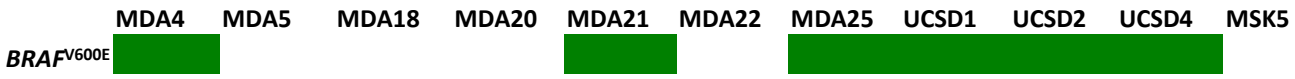
- **Age at diagnosis**: 49 years (15-76)

Results: at least one valid result was obtained in 33 of 38 (87%) patients

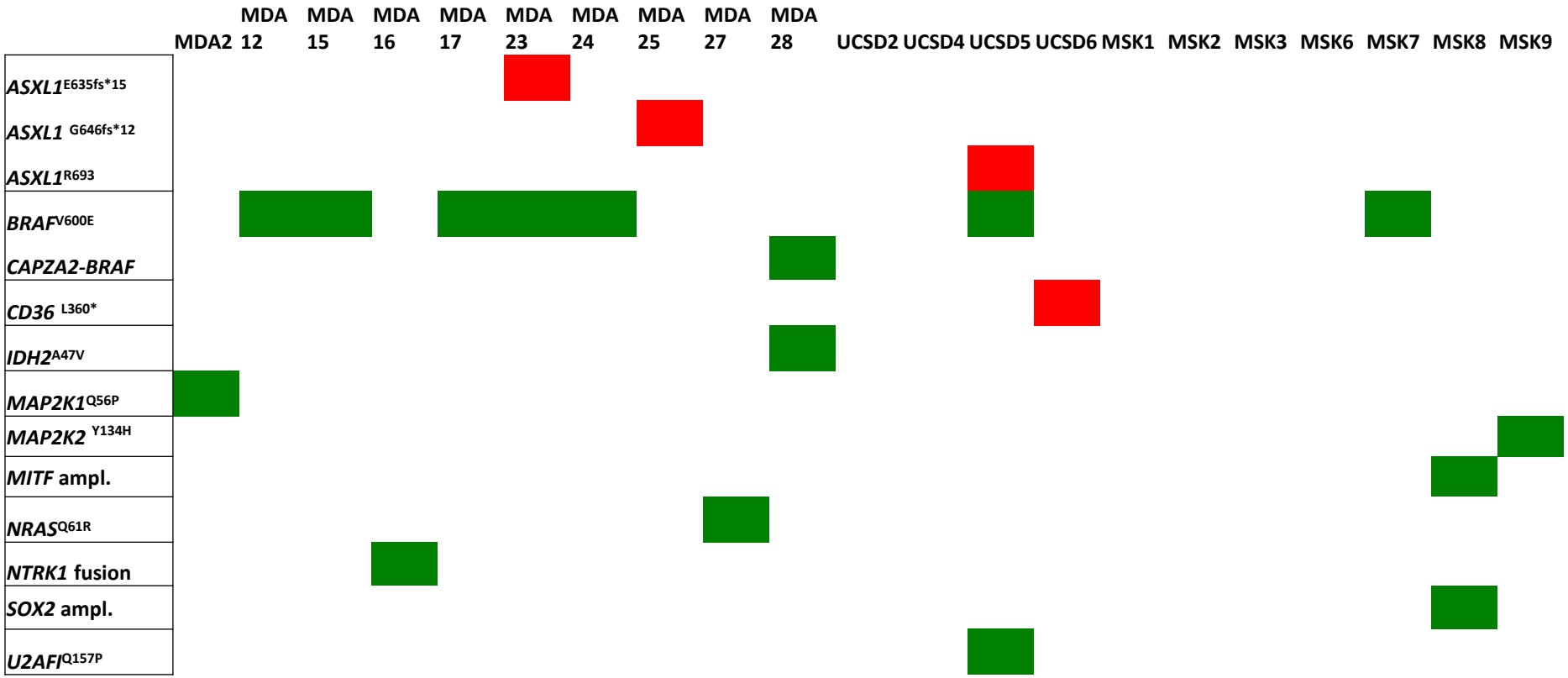
- **Tumor tissue PCR**: 15 patients
  - Molecular testing successful in 11/15 (73%)
- **Tumor tissue targeted NGS**: 29 patients
  - Molecular testing successful in 21/29 (72%)
- **Plasma cfDNA targeted NGS**: 26 patients
  - Molecular testing successful in 26/26 (100%)
- **Urine cfDNA PCR**: 4 patients
  - Molecular testing successful in 4/4 (100%)

Detected Molecular Alterations: HEATMAPS

PCR of Tumor Tissue

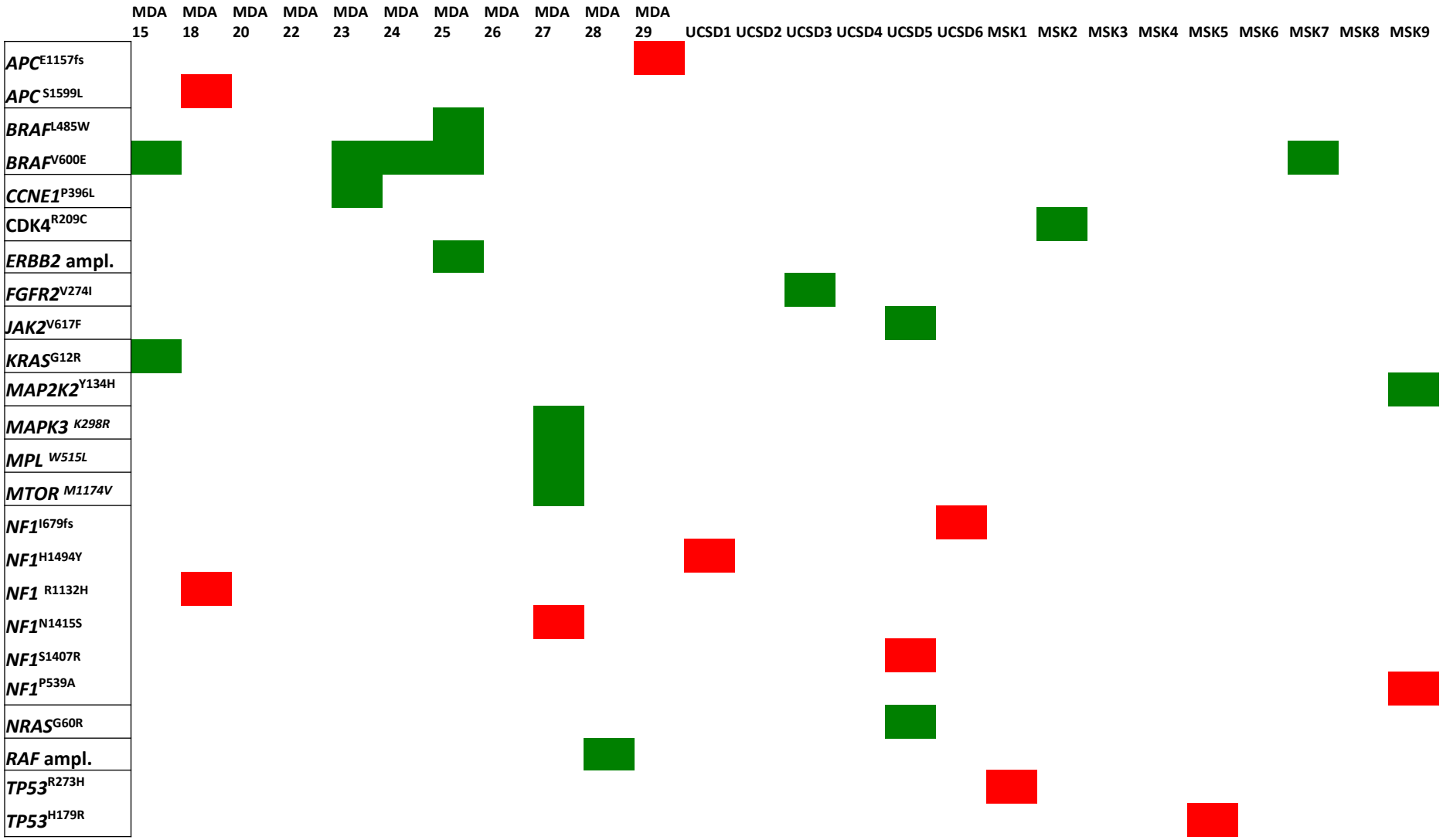


Targeted NGS of Tumor Tissue



Detected Molecular Alteration: HEATMAPS

Plasma NGS of Tumor Tissue



Detected Molecular Alteration: Frequencies

- **Tumor tissue PCR**: 11 patients with results
  - *BRAF*^{V600E}: 6 (54%)
- **Urine cfDNA PCR**: 4 patients with results
  - *BRAF*^{V600E}: 1 (25%)
- **Tumor tissue targeted NGS**: 21 patients with results
  - *BRAF*^{V600E}: 7 (33%)
  - *ASXL1* mutation: 3 (14%)
  - MAPK pathway alterations other than *BRAF*^{V600E}: 4 (19%)
- **Plasma cfDNA targeted NGS**: 26 patients with results
  - *BRAF*^{V600E}: 4 (15%)
  - *NF1* mutation: 6 (23%)
  - *APC* mutation: 2 (8%)
  - MAPK pathway alterations other than *BRAF*^{V600E} or *NF1*: 6 (23%)

Results: concordance tissue and plasma cfDNA

- **Total of 21 patients had valid results from molecular testing of tumor tissue DNA and cfDNA**
  - Targeted NGS of tumor and targeted NGS of plasma cfDNA: 14
  - PCR of tumor and targeted NGS of plasma cfDNA: 3
  - PCR and targeted NGS of tumor and targeted NGS of plasma cfDNA: 4

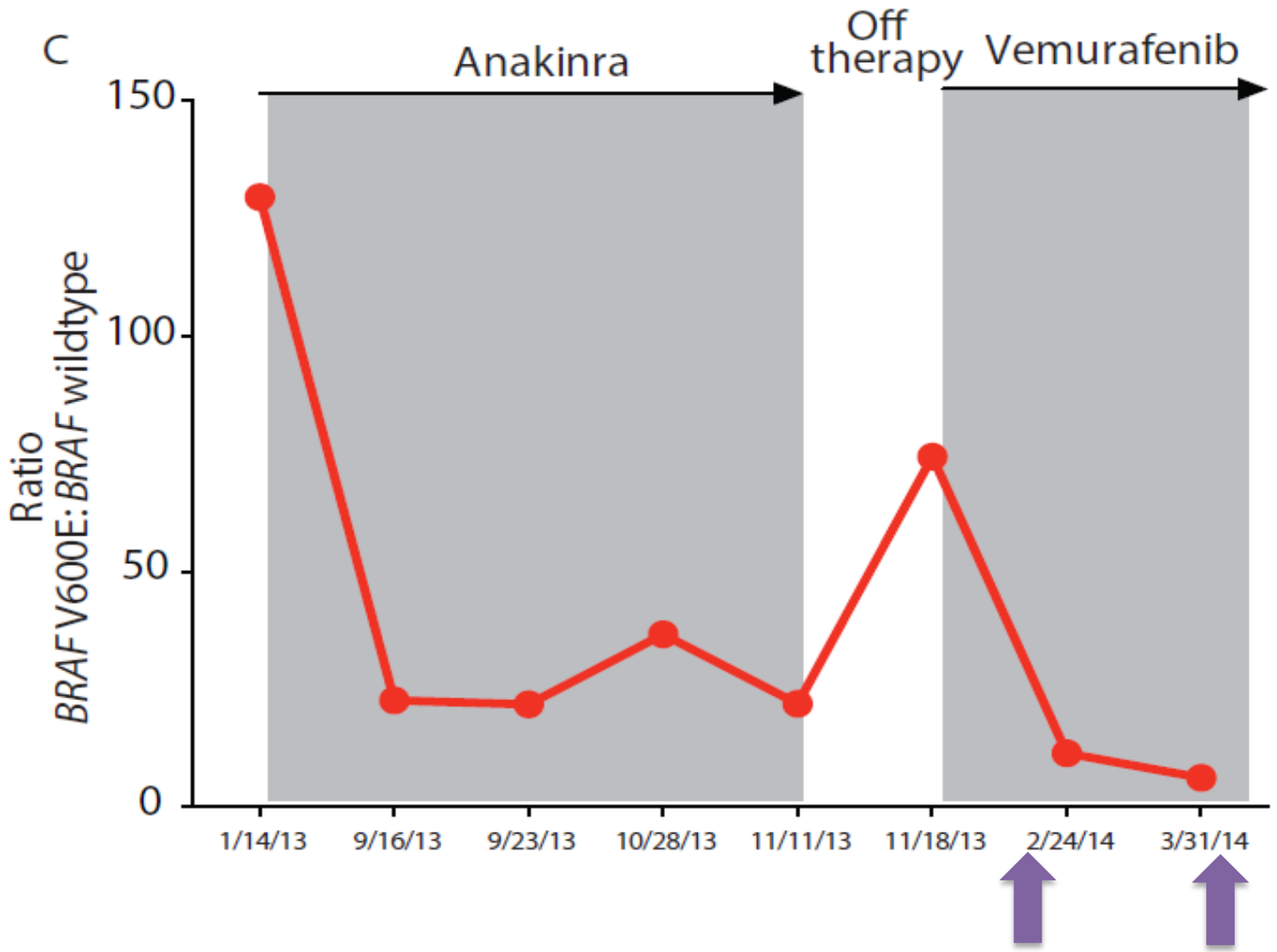
- **Targeted NGS of plasma cfDNA vs. targeted NGS of tumor DNA: 17**
  - Complete agreement: 6
  - Partial agreement: 3
  - Disagreement: 8

- **Targeted NGS of plasma cfDNA vs. PCR of tumor: 6**
  - Agreement: 3
  - Disagreement: 3

Discrepancies

Case ID	Tissue PCR	Tissue NGS	Plasma NGS
MDA15	N.D.	*BRAF*^{V600E}	*BRAF*^{V600E}, *KRAS*^{G12R}
MDA23	N.D.	*BRAF*^{V600E}, *ASXL1*^{E635fs*15}	*BRAF*^{V600E}, *CCNE1*^{P396L}
MDA25	*BRAF*^{V600E}	*ASXL1*^{G646fs*12}	*BRAF*^{V600E}, *BRAF*^{L485W}, *CCNE1*^{P396L}, *ERBB2* amplification
MDA27	N.D.	*NRAS*^{Q61R}	*MPL*^{W515L}, *MAPK3*^{K298R}, *NF1*^{N1415S}, *mTOR*^{M1174V}
MDA28	N.D.	*CAPZA2-BRAF* fusion, *IDH2*^{A47V}	*RAF1* amplification
UCSD1	*BRAF*^{V600E}	N.D.	*NF1*^{H1494Y}
UCSD2	*BRAF*^{V600E}	none	none
UCSD4	*BRAF*^{V600E}	none	none
UCSD5	N.D.	*BRAF*^{V600E}, *ASXL1*^{R693}, *U2AF1*^{Q157P}	*JAK2*^{V617F}, *NF1*^{S1407R},*NRAS*^{G60R}
UCSD6	N.D.	*CD36*^{L360*}	*NF1*^{I679fs}
MSK1	N.D.	none	*TP53*^{R273H}
MSK2	N.D.	none	*CDK4*^{R209C}
MSK8	N.D.	*SOX2* ampl., *MITF* ampl.	none
MSK9	N.D.	*MAP2K2*^{Y134H}	*MAP2K2*^{Y134H}, *NF1*^{P539A}

63-yo patient with Erdheim-Chester histiocytosis treated with BRAF inhibitor vemurafenib (ddPCR of urine cfDNA)



Hyman, Diamond, Janku, Abdel-Wahab. Cancer Discov 2014

DISCREPANCIES TISSUE vs. PLASMA

- ***BRAF*^{V600E}**
  - 4 plasma cfDNA samples were false negative and 3 of these 4 samples were collected during therapy with BRAF and/or MEK inhibitors

- **Timing of plasma collection**
  - Of 17 plasma cfDNA samples collected from patients with available treatment data, 7 (41%) were collected during systemic therapy

Results: turnaround times

The median turnaround times

- Tumor tissue PCR: 9 (5-41) days
- Tumor tissue targeted NGS: 21 (12-116) days
- Plasma cfDNA NGS: 13(8-18) days
- Urine cfDNA: 16.5 (7-25) days

Results: therapeutic implications

- 33 patients had at least one or more successful molecular testing
- 20 of 33 patients (61%) had targetable molecular alterations
- 13 of 20 patients (65%) received appropriate targeted therapy

CONCLUSIONS

- Clinical molecular testing in patients with non-Langerhans histiocytosis (mostly ECD) identifies targetable molecular alterations in nearly 2/3 of patients.

- Targeted NGS of plasma cfDNA has higher success rates and short turnaround times compared to tumor targeted NGS; however, agreement between methods are between 35%-53%. Agreement rates could be lower for plasma samples collected on therapy.