Novel recurrent mutations in Erdheim-Chester Disease patients identified by whole exome sequencing and whole genome sequencing

ECD Medical Symposium 2021

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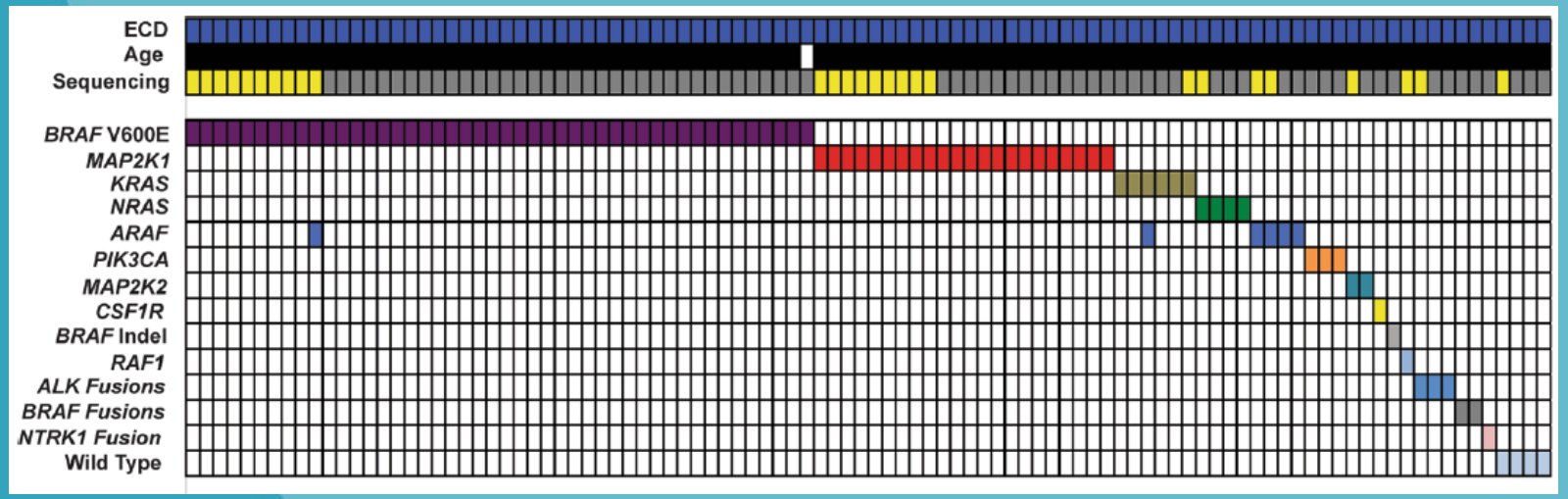
Background

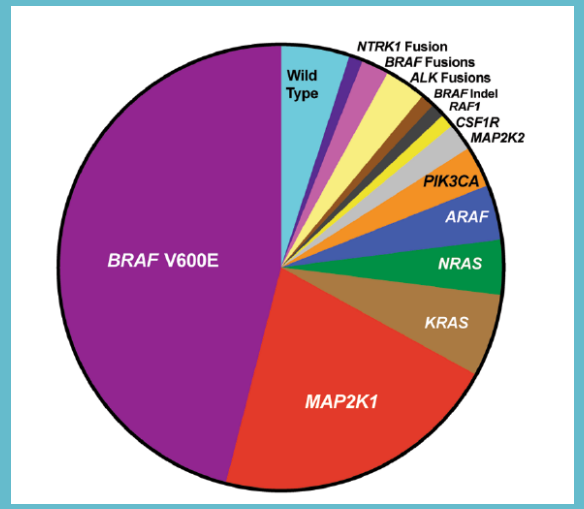
- Target therapies have great efficacy for many ECD patients, but treatment for patients without typical driver mutations are often difficult due to lack of specific target

- In treatment for other cancer like melanoma, BRAF or MEK inhibitors are supposed to develop secondary cancer

- There are no curative therapy for ECD

Known driver mutations in ECD





Age

- Pediatric
- Adult

Sequencing Analyses

- Whole exome sequencing and/or whole transcriptome sequencing
- Targeted DNA and/or RNA Sequencing

- WES were performed for 27 cases of ECD, and target-sequencing for 73 cases
- Four cases have no known driver mutation

Benjamin H. Durham. et al. Nature medicine 2015

Purpose

- Detect novel driver mutations in ECD patients
- Detect candidates for new target of treatment

Method

- We conducted nationwide survey for ECD in Japan, and collected clinical information about 54 ECD patients

  Takashi Toya. et al. Haematologica 2018

- Whole exome sequencing or whole genome sequencing for 21 samples of 14 ECD cases were performed

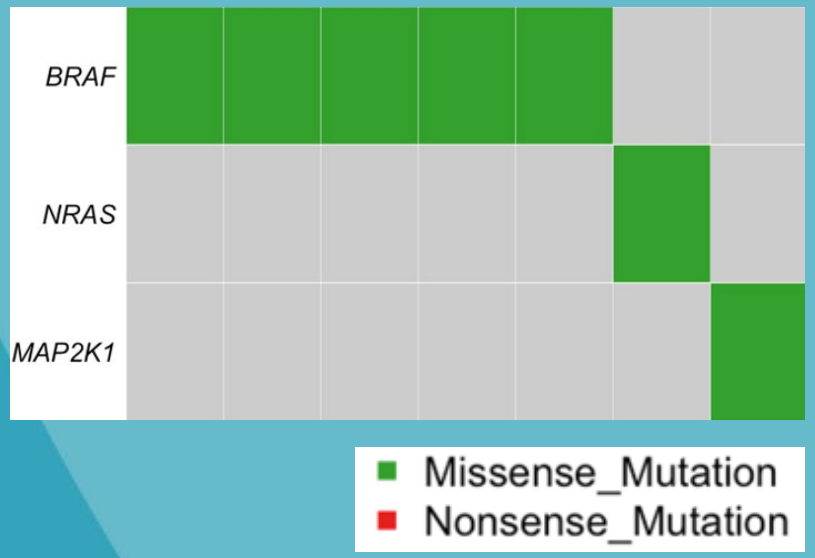
Result

- WES were performed for 14 samples of 14 ECD cases at diagnosis
- Characteristics of patients

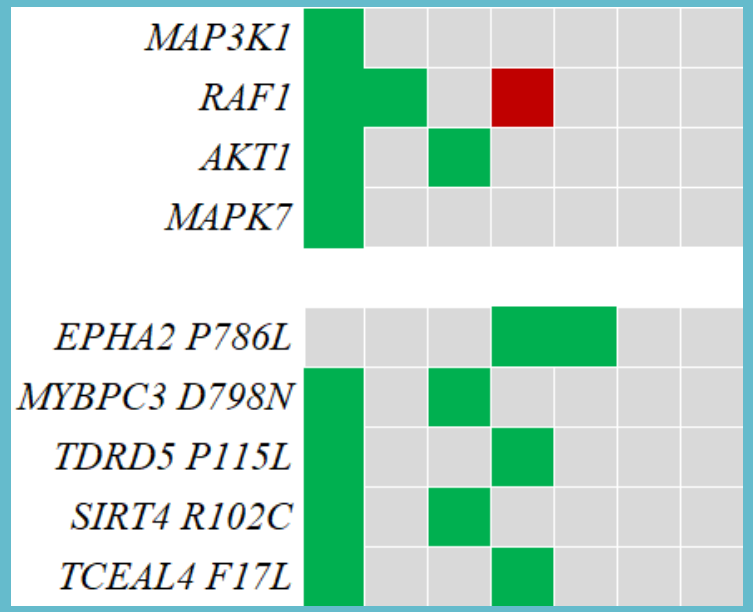
	n=14
Sex, M/F	7/7
Age at diagnosis (mean, SD)	48.9(13.7)
Mixed histiocytosis, (%)	1(7.1)
Bone involvement, (%)	13(92.9)
Cardiac/Vascular involvement, (%)	8(57.1)
Xantheasma, (%)	7(50.0)
Endocrine, (%)	7(50.0)
CNS involvement, (%)	4(28.6)
Retroperitoneal involvement, (%)	10(71.4)
Myeloid malignancies, (%)	1(7.1)

WES for newly diagnosed ECD cases

Cases with validated point mutations
in driver genes in ECD (n=7)



The other cases (n=7)



The left oncoplot shows driver kinase mutations validated in previous reports.
The right oncoplot is about 7 patients without validated driver mutations.
This shows possible driver mutations and recurrent point mutations in these cases.
Notably, *EPHA2* P786L mutation is mutually exclusive with cases in which validated or possible driver mutations are detected.

Candidates of novel driver mutations

Gene	Variant type	Protein change	Function	Clinvar	PolyPhen2
EPHA2	SNP	P786L	Receptor tyrosine kinase	not reported	Probably damaging(1)
MYBPC3	SNP	D798N	Myosin binding protein	not reported	Possibly damaging(0.9)
TDRD5	SNP	P115L	Spermatogenesis	not reported	Possibly damaging(0.4)
SIRT4	SNP	R102C	Cancer suppressor	Benign	Possibly damaging(1)
TCEAL4	DNP	F17L	Transcription elongation factor	N/A	N/A

EPHA2 is a receptor tyrosine kinase, and P786L mutation occur in the kinase domain. Thus, we focus on *EPHA2* P786L mutation.

EPHA2 (Ephrin type-A receptor 2) in cancer and inflammation

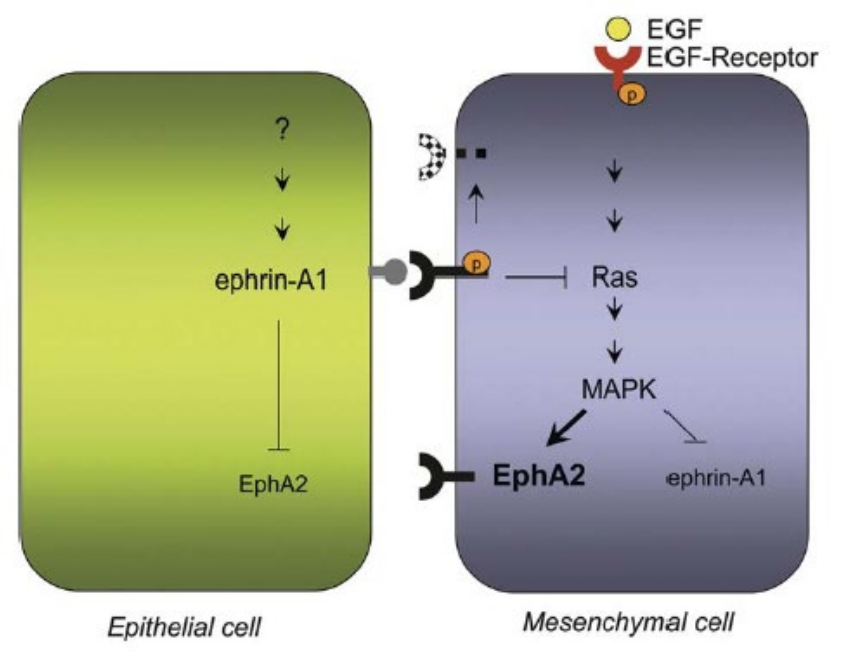
EPHA2 is abundantly expressed in diverse cancers. EPHA2 expression has associations with poor prognosis, elevated metastatic potential, and reduced survival of tumor patients. Ta Xiao. et al. Journal of Hematology & Oncology 2020

EPHA2 mutations with oncogenic characteristics in squamous cell lung cancer and malignant pleural mesothelioma. Yi-Hung Carol Tan. et al. Oncogenesis 2019

EPHA2 functions as a β-glucan receptor that triggers the production of proinflammatory mediators in response to oropharyngeal candidiasis.
Marc Swidergall. et al. Cell reports 2019

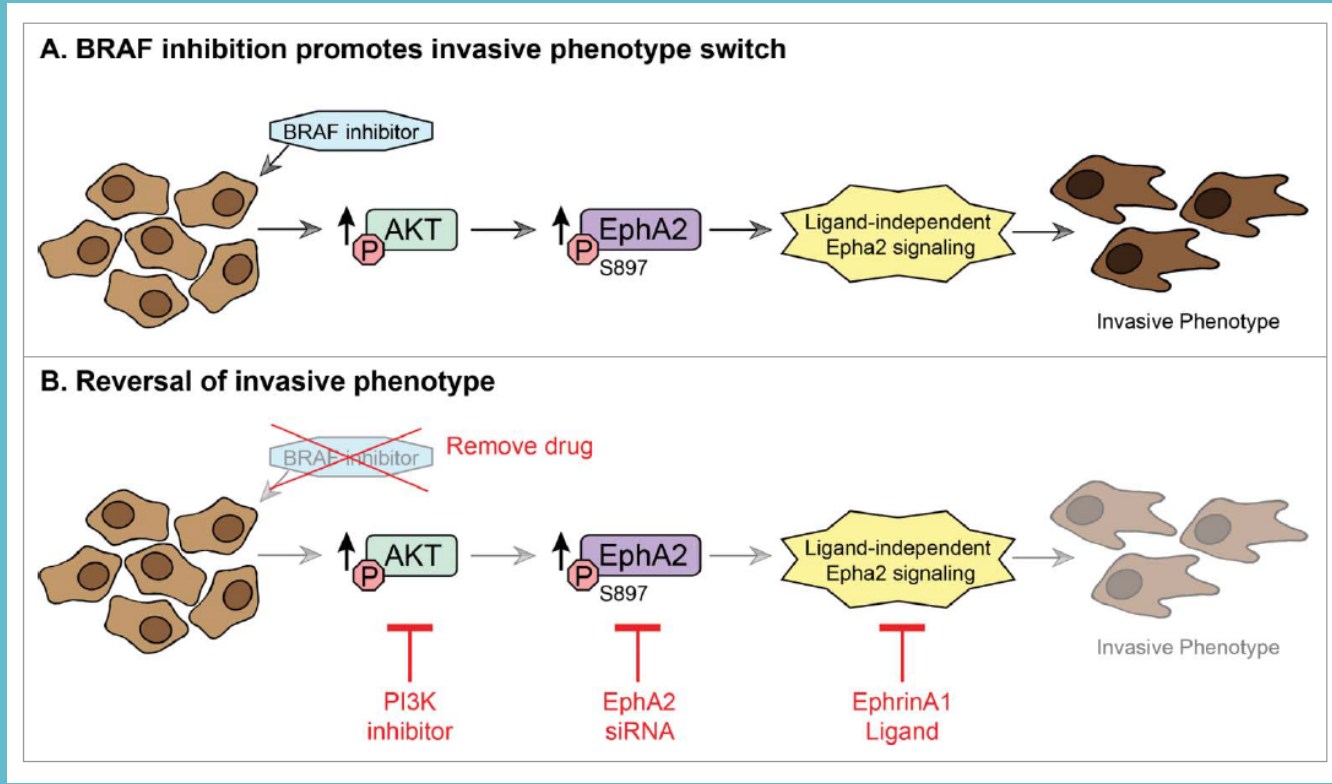
EPHA2 in MAPK and PI3K-AKT pathways

EPHA2 is upregulated by pERK in breast cancer



Madhu Macrae. et al. Cancer Cell 2005

EPHA2 is activated ligand-independently by pAKT in malignant melanoma



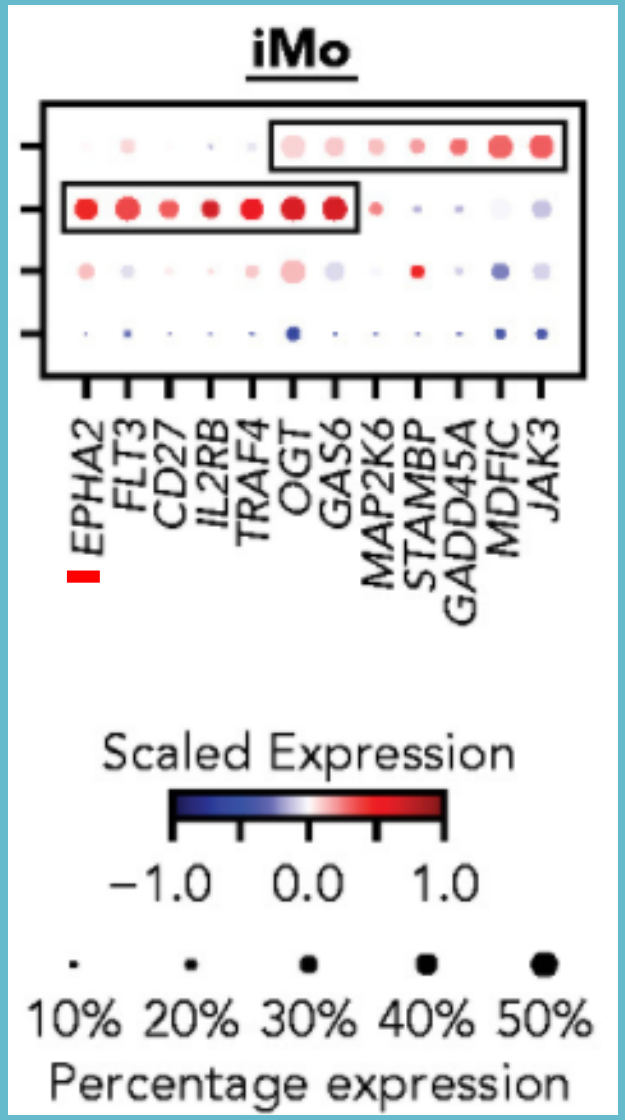
Keiran SM Smalley. et al. Molecular & Cellular Oncology 2015

EPHA2
in Histiocytic disorder

Transcriptomic landscape of circulating mononuclear phagocytes in Langerhans cell histiocytosis at the single-cell level

EPHA2 is significantly upregulated in immature monocyte in LCH cases

Hui Shi. et al. Blood 2021



Organ involvement associated with poor prognosis in ECD

TABLE 2 Predictors of poor survival in ECD (multivariate survival analysis using the Cox proportional hazard ratio)

Variable	Cox survival analysis		
	Univariate HR (95% CI)	Multivariate HR (95% CI)	*P*-value
Sex M[a]	1.15 (0.58; 2.31)	1.96 (0.89; 4.28)	.0932
Age at diagnosis (per year increase)[a]	1.05 (1.02; 1.08)	**1.06 (1.03; 1.09)**	**.0001**
$BRAF^{V600E}$	1.27 (0.54; 2.94)	1.73 (0.68; 4.41)	.2495
BRAF missing	2.02 (0.85-4.79)	2.02 (0.83; 4.94)	.1220
CNS involvement	1.37 (0.73; 2.55)	**2.62 (1.28;5.37)**	**.0084**
Cardiac involvement	1.36 (0.72; 2.57)		
Lung involvement	3.01 (1.58; 5.75)	**2.74 (1.38; 5.43)**	**.0038**
Vascular involvement	1.27 (0.64; 2.50)		
Xanthelasma	0.74 (0.35; 1.56)		
Diabetes insipidus	0.43 (0.18; 1.04)		
Retroperitoneal involvement	2.10 (1.05; 4.22)	**3.85 (1.68; 8.83)**	**.0014**
IFN-alpha treatment	0.56 (0.26; 1.19)	**0.38 (0.16; 0.89)**	**.0257**
Targeted therapy	0.41 (0.16; 1.06)	**0.33 (0.11; 0.93)**	**.0364**

[a]Adjustment variables.
CNS, central nervous system; IFN, interferon.

Fleur. et al.
Am J Hematol. 2018

CNS, lung, and retroperitoneal involvement are predictors of poor survival.
There might be correlations in mutations and organs involved.
Mutated genes associated with these organs might be new targets of treatment.

About organ-specific mutation

- We could not detect organ-specific mutation by these 14 cases
- We performed intra-patient analysis of a case with multiple organ lesions and concurrent hematologic malignancies

Case. 57 Female

- 49 y.o. Diagnosed as ECD with bone biopsy→Bone lesion
  - Treated with IFN-α, but discontinued due to a side effect

- 56 y.o. Diagnosed as MDS→BM with MDS
  - Treated with Azacytidine 2 cycles
- 57 y.o. Subsequently developed AML→ BM with AML
  - Treated with Intermediate Cytarabine 2 cycles
    →Complete Response

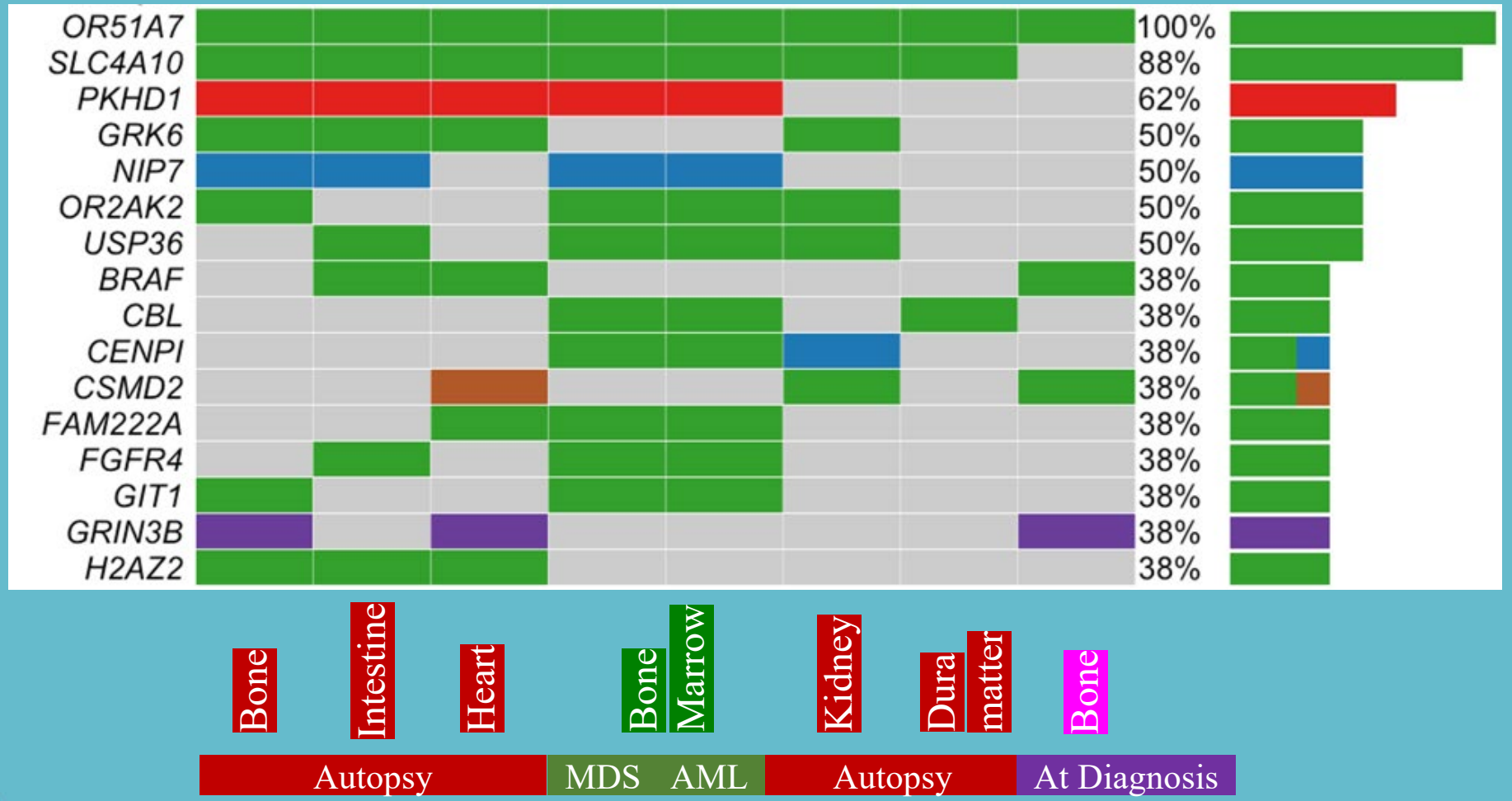
- Day 23 of the 2nd cycle of the chemotherapy, she died of subarachnoid hemorrhage (after cerebral stroke)
  - Autopsy→Bone, intestine, heart, kidney and dura matter lesions

Mutational profiles of multiple lesions

OR51A7 mutation is detected in all samples

BRAF V600E is found in 3 samples

IRGC, *RASSF6*, and *EZH2* mutations are detected only in MDS/AML samples



Conclusions

- *EPHA2* P786L, *MYBPC3* D798N, *TDRD5* P115L, *SIRT4* R102C, and *TCEAL4* F17L are recurrently found in newly diagnosed ECD
- Mutational profiles of multiple organ lesions in an ECD case can differ from each other
- Further research for new target of treatment and organ-specific mutations are warranted

Acknowledgements

THE UNIVERSITY OF TOKYO

Department of Pathology,
Graduate School of Medicine
Aya Shinozaki-Ushiku

Department of Rehabilitation Medicine,
Graduate School of Medicine
Yusuke Shinoda

Osaka University Hospital
Keio University Hospital
Juntendo University Hospital
Okayama University Hospital
Niigata University Medical and Dental Hospital
Kagoshima University Hospital
Hamamatsu University School of Medicine
Hokaido Cancer Center
National Hospital Organization Kyushu Cancer Center
Kochi Health Siences Center
National Hospital Organization Higashihitoshima Medical Center
Japanese Red Cross Otsu Hospital
Tsuchiura Kyodo General Hospital

Funding Source

AMED
Japan Agency for Medical Research and Development