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Plasma-Derived Cell-Free DNA for the Diagnosis of Ocular-Involving Histiocytosis

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Purpose: Circulating tumor DNA (ctDNA) is released into the plasma by many cancers and offers clinical applications including noninvasive diagnostics. Histiocytosis results from myelogenous clonal expansion of histiocytes, predominantly driven by mutations in the mitogen-activated protein kinase pathway that are potentially detectable by ctDNA-based sequencing assays. However, ocular-involving histiocytosis is often a diagnostic challenge leading to delayed diagnosis and the need for invasive biopsy of sensitive ocular structures. The purpose of this study is to determine whether sequencing of plasma-derived ctDNA can noninvasively diagnose ocular-involving histiocytosis.

Design: Single tertiary cancer referral center.

Participants: Twenty-four adult patients with ocular-involving histiocytosis and ctDNA sequencing.

Methods: Circulating tumor DNA was analyzed (via digital droplet polymerase chain reaction for BRAF V600E, and/or next-generation sequencing) and variant allele frequency was measured at initial presentation to our center. Patient demographics, clinical characteristics, and oncogenic mutations identified from tumor-based sequencing were recorded.

Main Outcome Measures: Plasma-derived ctDNA detectability of pertinent driver mutations of histiocytosis.

Results: At the initial presentation of 14 patients with ocular-involving histiocytosis, sequencing of plasma-derived ctDNA detected driver mutations for histiocytosis (BRAF V600E [10], KRAS [2], ARAF [1], and concurrent MAP2K1/KRAS [1]). Mutations found in circulating cell-free DNA were 100% concordant in 11 of 11 patients with mutations identified by solid tumor sequencing. Of 10 patients without driver mutation detected in ctDNA, 3 patients had alterations (CBL mutation or kinase fusion) not captured in the ctDNA sequencing assay, 3 were wildtype even by tumor sequencing; in 4 patients, tumor-based sequencing identified mutations (BRAF [2], MAP2K1 [2]) not detected in ctDNA. Detectable mutations in ctDNA were significantly more likely in patients with uveal infiltration ($P = 0.036$).

Conclusions: In this cohort, plasma-derived ctDNA was detectable and diagnostic in the majority of patients with ocular-involving histiocytosis. This suggests that if ocular histiocytosis is suspected (particularly if involving the uvea), noninvasive plasma-derived ctDNA analysis is a helpful diagnostic tool that may obviate the need to invasively biopsy sensitive ocular structures.

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Circulating cell-free DNA (cfDNA) that is released from tumor cells can have clinical applications for cancer.[1,2] Circulating tumor DNA (ctDNA) is a small component of cfDNA, specifically sourced from tumor cells.[1,2] Through identification of the driver mutation, ctDNA has emerged as a noninvasive, easily repeatable diagnostic tool for many malignancies. Variant allele frequency, a proxy for tumor fraction, provides crucial longitudinal data allowing for treatment response measurements, which may subsequently guide management. Applications for plasma ctDNA are ongoing in retinoblastoma[3] and uveal melanoma[4] but are otherwise understudied in other ocular diseases, particularly in ocular-involving histiocytosis.

Histiocytoses are rare, myeloid-derived clonal hematopoietic disorders in which there is an accumulation of histiocytes and infiltration of tissues. Subtypes include Langerhans cell histiocytosis (LCH; L-group histiocytosis) and non-Langerhans-cell histiocytoses (non-LCH), the latter of which includes Rosai-Dorfman-Destombes disease (RDD, R-group histiocytosis), Erdheim-Chester disease (ECD; L-group histiocytosis), and numerous xanthogranuloma (C-group) disorders. A recent pivotal discovery showed disease pathophysiology is driven by recurrent activating mutations in the mitogen-activated protein kinase (MAPK) pathway in the majority of L-group histiocytosis patients[5–7] and then in RDD and others.[8] This led to a major therapeutic breakthrough involving long-term responses with targeted agents to these activating mutations, such as BRAF-inhibitors and MEK-inhibitors.[9–11] As we embark on this investigation, we note that 30% to

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50% of the RDD subtype do not have identifiable mutations despite comprehensive tumor sequencing and therefore any form of sequencing may not be as useful for this subtype.

Histiocytoses are clinically heterogeneous, manifesting as localized or diffuse multisystem disease. The most severe cases involve critical anatomical sites such as ophthalmic structures, which are present in 54% of our histiocytosis cohort (Francis JH, unpublished data, 2024). Histiocytoses often have delayed diagnosis due to their propensity to mimic other infiltrating inflammatory or malignant conditions. The masquerading nature of histiocytosis, combined with the need to invasively biopsy potentially sensitive anatomic areas (such as the choroid), renders ocular histiocytosis a diagnostic challenge. To this point, recent guidelines for LCH[12] and ECD[13] highlight the contribution of cfDNA testing when tumor material is limited. In support of this, cfDNA has been shown to be highly concordant with tumor sequencing in BRAF-mutant histiocytosis[14] and variable concordance in BRAF-wildtype histiocytosis.[15] In this study, we investigate whether ctDNA analysis can address the unmet need of noninvasively diagnosing ocular histiocytosis.

Methods

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center. This retrospective, single-center study included 24 eligible patients recruited from Memorial Sloan Kettering Cancer Center, New York between January 2014 and September 2021. Each patient gave informed consent and eligible patients had findings suspicious for ocular-involving histiocytosis and ctDNA plasma testing at presentation to our center.

Plasma ctDNA was analyzed by either digital droplet polymerase chain reaction (ddPCR) of BRAF V600E and/or next-generation sequencing (NGS) using MSK-ACCESS, an assay using deep sequencing and hybridization capture to detect very low-frequency somatic alterations in 129 cancer-related genes. These assays are approved for clinical use by the New York State Department of Health. The MSK-ACCESS assay can detect single nucleotide polymorphisms, insertions or deletions of bases (Indels), and copy number alterations. Variant allele frequencies (the proportion of reads bearing the variant divided by the total number of reads at a given genomic location) $\geq$0.1% were recorded. Matched sequencing of bulk white blood cell cellular DNA was performed to identify and filter both germline variants and mutations associated with clonal hematopoiesis.[16]

For evaluation of tumor mutations, genomic DNA was extracted from formalin-fixed paraffin-embedded samples and underwent mutational analysis using previously described techniques. This included allele-specific polymerase chain reaction for BRAFV600E, whole-exome sequencing, or Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets for Hematological Neoplasms, a targeted hybrid capture NGS-based DNA sequencing panel of 468 cancer genes with a matched-normal sample to exclude germline variants.[7,17,18] For some patients, an NGS-based RNA-fusion detection assay (Archer FusionPlex Custom Solid Panel) was used to detect fusions across 123 cancer-related genes involved in chromosomal rearrangements.[19]

The following data points were recorded: patient characteristics (gender, age at diagnosis), tumor characteristics (histiocytosis subtype, other sites of disease), ocular structures involved, and treatment modalities. Genetic data included the mode of ctDNA analysis (ddPCR or NGS), detected genes and their variant allele frequency, and the site of solid tumor that provided pathological evidence of histiocytosis.

Categorical data were compared using the 2-tailed Fisher exact test with statistical significance set at a P value <0.05.

Results

Patient, disease, and ocular structure details of detectable ctDNA cases are shown in Table 1. Of 14 patients, 8 were male and the median age at diagnosis was 51.0 (22.1−79.4) years. The distribution of histiocytosis subtypes were as follows: ECD in 9, RDD in 2, xanthogranuloma in 1, and mixed ECD and LCH in 2. All but 1 patient had multisystemic histiocytosis. Half the patients had >1 ocular structure involved and the most common were as follows: choroid in 8, orbit in 4, and eyelid in 3. Figure 1 demonstrates representative images for 12 patients.

Table 1 shows genetic data related to cases with detectable ctDNA. Nine patient samples were tested by both the ddPCR and NGS assays and 5 were assessed by the NGS assay alone. Earlier specimens were mostly analyzed through ddPCR and then escalated to NGS if the former was negative. Specimens later in the study were mainly evaluated via NGS due to increased testing availability over time.

Circulating tumor DNA detected driver mutations in the following distribution: BRAF V600E in 10, KRAS in 2, ARAF in 1, and both MAP2K1/KRAS in 1. Molecular genetic data of solid tumor specimens were available for comparison in 11 patients: the detected genetics by ctDNA was 100% concordant in all cases. Detected mutations were implicated in the MAPK pathway and all 14 patients were subsequently treated with agents targeting this defunct pathway. Circulating tumor DNA demonstrating a MAPK mutation established a diagnosis of histiocytosis in 3 patients without diagnostic biopsy tissue, identified a MAPK pathway mutation in 9 patients with biopsy-proven histiocytosis without mutation identified by tumor sequencing at the time of ctDNA analysis, and was confirmatory in 2 patients with pathology, and genetically, established histiocytosis by prior outside genetic testing.

Table 2 shows patient, disease, and genetic data for cases in which ctDNA sequencing demonstrated no mutations. In this group, 3 were male, the median age was 48.6 (26.3−60.3) years, and the subtypes were distributed as follows: RDD (5), ECD (2), xanthogranuloma (1), ECD/LCH (1), ALK-positive histiocytosis (1); these variables were not statistically different from the ctDNA-detectable group. The ctDNA-detectable group had significantly more uveal disease (choroidal infiltration or uveitis) compared with the ctDNA-undetectable group (71.4% versus 20%, P value = 0.036). In the 10 ctDNA-undetectable patients, 6 were expected to be negative due to the known inability of the ctDNA assay to detect fusions in 2, CBL mutation in 1, and wildtype disease by any method in 3. There were negative ctDNA sequencing results

Table 1. Patient, Disease, Ocular Structure, and Genetic Data in Patients with Detectable Plasma ctDNA

Patient	Age at Diagnosis (Yrs)	Gender	Histiocytosis Subtype	Other Sites of Disease	Eye Findings	Mutational Status	cfDNA Testing	cfDNA VAF	Site of Solid Tissue Confirmation
1	55.1	M	ECD	Bone, brain, CV, pulmonary, retro, abdomen	Choroid, orbit	BRAF V600E	NGS, ddPCR	0.95%	Perirenal
2	52.5	M	ECD	Bone, brain, retro, abdomen	Choroid, dysmotility	BRAF V600E	NGS	0.85%	Perirenal and tibia*
3	47.8	M	XG	None	Orbit	KRAS K117N	NGS	0.07%	Orbit*
4	25.5	M	ECD/LCH	Bone, CV, skin	Sclera, orbit, eyelid	BRAF M484_P490 deletion	NGS, ddPCR	0.48%	Skin (eyelid)
5	79.4	F	RDD	Pulmonary, nasopharynx	Choroid, CN VI palsy	KRAS in-frame insertion Y64_S65insVS	NGS	3.48%	Nasopharyngeal
6	68.9	F	ECD	Bone, skin, LN	Choroid, eyelid	BRAF V600E	NGS, ddPCR	0.54%	Skin (eyelid)
7	47.1	F	ECD	Neuro (DI), bone, brain, CV, retro	Choroid	BRAF V600E	NGS, ddPCR	1.81%	Perirenal
8	49.6	F	RDD	Bone, retro, LN	Choroid	MAP2K1 K57T, KRAS A146P	NGS	0.37% KRAS, 0.12% MAP2K1	Lymph node
9	22.1	M	ECD/LCH	Neuro (dura), bone, skin	Orbit	BRAF V600E	NGS, ddPCR	0.46%	Temporal lobe
10	66.2	F	ECD	Neuro (dura), bone, brain, CV	Choroid	BRAF V600E	NGS, ddPCR	0.07%	Dura
11	37.8	M	ECD	Neuro (dura, pituitary), bone, brain, CV, skin, testes	Optic nerve	BRAF V600E	NGS, ddPCR	0.52%	Testes
12	67.7	M	ECD	Neuro (DI), bone, brain, CV, retro, skin	Sclerochoroidal, eyelid	BRAF V600E	NGS, ddPCR	0.06%	Skin (eyelid)
13	61.8	F	ECD	Bone	Choroid, orbit	ARAF S213A	NGS, ddPCR	0.89%	Bone
14	34.4	M	ECD	Neuro (DI), bone, brain, retro, LN	Uvea (uveitis)	BRAF V600E	NGS	0.08%	Lymph node*

cfDNA = cell-free DNA; CN = cranial nerve; ctDNA = circulating tumor DNA; CV = cardiovascular; ddPCR = digital droplet polymerase chain reaction; DI = diabetes insipidus; ECD = Erdheim-Chester disease; F = female; LCH = Langerhans cell histiocytosis; LN = lymph node; M = male; neuro = neurological; NGS = next-generation sequencing; RDD = Rosai-Dorfman disease; retro = retroperitoneum; VAF = variant allele frequency; XG = xanthogranuloma.
*No available molecular genetics of solid tumor.

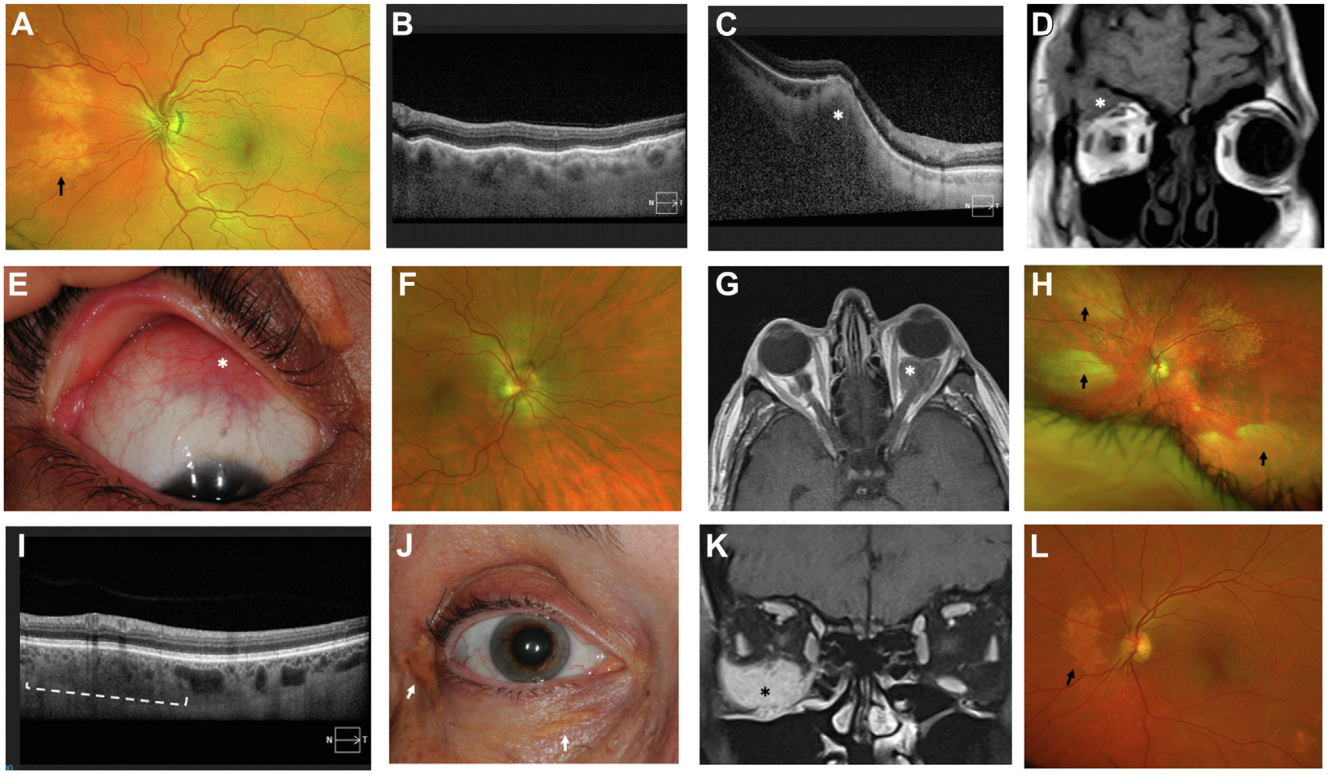

Figure 1. Representative patient images. **A,** Color fundus photo of choroidal disease in Erdheim-Chester Disease (ECD) patient 7 (arrow). **B,** OCT of choroidal disease in ECD patient 10 showing undulating choroid. **C,** OCT of sclerochoroidal disease in ECD patient 12 (asterisk). **D,** Magnetic resonance imaging (MRI) of orbital disease in ECD/Langerhans cell histiocytosis (LCH) patient 9 (asterisk). **E,** External photo of episcleral disease in ECD/LCH patient 4 (asterisk). **F,** Color fundus photo of optic nerve infiltration in ECD patient 11 (arrows). **G,** MRI of orbital disease in ECD patient 1 (asterisk). **H,** Color fundus photo of choroidal disease in RDD patient 5 (arrows). **I,** OCT of choroidal disease in RDD patient 8 (white dashed line). **J,** External photo of eyelid disease infiltrating disease mimicking xanthelasmas in ECD patient 6 (arrows). **K,** MRI of orbital disease in Xanthogranuloma patient 3 (asterisk). **L,** Color fundus photo of choroidal disease in ECD patient 2 (arrow).

in 2 patients with BRAF and 2 with MAP2K1 alterations: 2 of these were found by tumor sequencing methods described earlier and 1 was found only on a urine-based ctDNA assay previously validated in histiocytosis.[14] On the basis of their mutational analysis, patients were initiated on agents targeting the MAPK pathway, including BRAF and MEK: vemurafenib (3), trametinib (1), dabrafenib/trametinib (6), cobimetinib (6), and vemurafenib/cobimetinib (1).

Discussion

By demonstrating the detectability of plasma-derived ctDNA of mutations driving histiocytosis in 14 patients, we illustrate ctDNA as a crucial noninvasive diagnostic tool in ocular-involving histiocytosis, which has treatment implications by identifying appropriate mutation-specific targeted agents. This is particularly pertinent to patients with disease infiltrating sensitive structures of the eye, such as the choroid, one of the most commonly implicated structures occurring in half of our patients with detectable ctDNA and in isolation of other ocular structures in 30% of patients. To this point, patients with detectable ctDNA had a statistically significant predominance of uveal disease (choroid infiltration or uveitis) compared with patients with undetectable ctDNA (P value $= 0.036$). Therefore, rather than pursue an invasive, potentially risky intraocular biopsy that requires multidisciplinary coordination, the diagnosis may be captured through a noninvasive, office-based blood test. One patient has isolated ocular disease with positive ctDNA, demonstrating that the assay can successfully detect mutations in the absence of multisystemic disease.

As part of establishing the feasibility of this liquid biopsy, we compared mutational results with the gold standard of solid tumor molecular profiling. In 11 of 14 patients with available solid tumor genetic testing, the mutational profile was the same as detected by blood-based ctDNA, demonstrating 100% concordance between liquid and solid biopsies. In 3 patients, molecular profiling of solid tumor specimens was negative and ctDNA represented the only means of establishing the diagnosis and identifying the driver mutation. Therefore, in cases where solid tumor profiling is inconclusive, ctDNA has an important role in mutational profiling not only for diagnostics but also in identifying mutations that are amenable to therapeutic targeted agents. All 14 patients were appropriately managed on agents targeting the respective pathway driven by their pathological mutation.

Table 2. Patient, Disease, Ocular Structure, and Genetic Details in Patients with Undetectable Plasma ctDNA

Patient	Age at Diagnosis (Yrs)	Gender	Histiocytosis subtype	Other Sites of Disease	Eye Findings	Mutational Status	Negative cfDNA Expected or Unexpected
15	60.3	m	RDD	Neuro (dura), bone, retro, abdomen	Optic nerves	MAP2K1 E203K	Unexpected
16	26.3	m	RDD	Brain	Sclera, cornea, optic nerves	Unknown	Expected
17	52.8	f	ECD	Bone, brain	Optic nerves	BRAF V600E	Unexpected*
18	45.8	f	XG	Retro, breast	Orbit	Unknown	Expected
19	55.7	f	ECD	Bone, CV, pulmonary, abdomen, skin	Sclera	CBL	Expected
20	20.2	m	ECD/LCH	Bone, skin	Uvea, vitreous	BRAF V600E	Unexpected
21	51.4	f	RDD	CV, skin, sinus	Sclera, choroid, uvea	MAP2K1 Y130C	Unexpected†
22	10.2	f	RDD	Bone, brain, LN	Optic nerves	Unknown	Expected
23	55.8	f	RDD	Bone, skin, laryngeal, breast	Sclera	ETV6-SYK fusion	Expected
24	28.4	f	ALK-positive	Bone, brain, LN	Orbit	KIF5B-ALK Fusion	Expected

cfDNA = circulating cell-free DNA; CN = cranial nerve; ctDNA = circulating tumor DNA; CV = cardiovascular; DI = diabetes insipidus; ECD = Erdheim-Chester disease; f = female; LCH = Langerhans cell histiocytosis; LN = lymph node; m = male; neuro = neurological; RDD = Rosai-Dorfman disease; retro = retroperitoneum; XG = xanthogranuloma.
*Mutation found on urine-based ctDNA assay.
†Mutation found on research-based whole-exome sequencing.

The mutational profile of the cohort reflects known mutations in this clinical population, namely, BRAF, ARAF, KRAS, and MAP2K1. However, even rare mutations such as CBL or MAP2K2 (occurring in <1% of patients) or fusions (occurring in 5%−10% of patients), would not be identifiable through our current NGS ctDNA assay (but potentially possible with other ctDNA assays), and instead would necessitate detection through NGS of solid tumor or RNA sequencing for fusion analysis. Finally, approximately 30% to 50% of RDD patients have wildtype disease (although, interestingly, may still be amenable to targeted inhibition of the MAPK pathway)[20]; and in this instance, ctDNA would not be diagnostically useful. In the entire cohort of 24 patients, ctDNA was identifiable in 58% patients (and undetectable in 42%); however, given the known limitations of our ctDNA assay (inability to capture fusions or exclusion of rarer mutations on the panel) and the potential for mutation-free histiocytosis (particularly RDD and xanthogranuloma), detectable ctDNA was expected in 18 of 24 (75%) in our cohort. Our results show that ctDNA was detectable in 14 of 18 (78%) of these patients with ocular-involving histiocytosis.

In summary, we demonstrate that blood-based ctDNA has a role in ophthalmology for the diagnosis of ocular-involving histiocytosis. If clinical features are suspicious for histiocytosis, this noninvasive blood test may be diagnostic and may obviate the need for potentially risky biopsy of sensitive ocular structures such as the choroid, sclera, and optic nerves, all of which have been implicated in histiocytosis. Even though this study used an institutional ctDNA assay, there are a growing number of commercially based assays broadly accessible to the ophthalmic provider community. We believe this diagnostic modality is a powerful tool in challenging ocular-involving histiocytosis in which there is a diagnostic dilemma, and hope this proves to be true on a larger scale.

Footnotes and Disclosures

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sponsor or funding organization had no role in the design or conduct of this research.

HUMAN SUBJECTS: Human subjects were included in this study. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center. This retrospective, single-center study included 24 eligible patients recruited from Memorial Sloan Kettering Cancer Center, New York between January 2014 and September 2021. Each patient gave informed consent and eligible patients had findings suspicious for ocular-involving histiocytosis and circulating tumor DNA plasma testing at presentation to our center.

No animal subjects were used in this study.

Author Contributions:

Conception and design: Francis, Diamond

Data collection: Francis, Arcila, Sigler, Bossert, Diamond

Analysis and interpretation: Francis, Arcila, Abramson, Diamond

Obtained funding: Francis, Diamond

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Abbreviations and Acronyms:
cfDNA = circulating cell-free DNA; **ctDNA** = circulating tumor DNA; **ddPCR** = digital droplet polymerase chain reaction; **ECD** = Erdheim-Chester disease; **LCH** = Langerhans cell histiocytosis; **MAPK** = mitogen-activated protein kinase; **NGS** = next-generation sequencing; **RDD** = Rosai-Dorfman-Destombes disease.

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