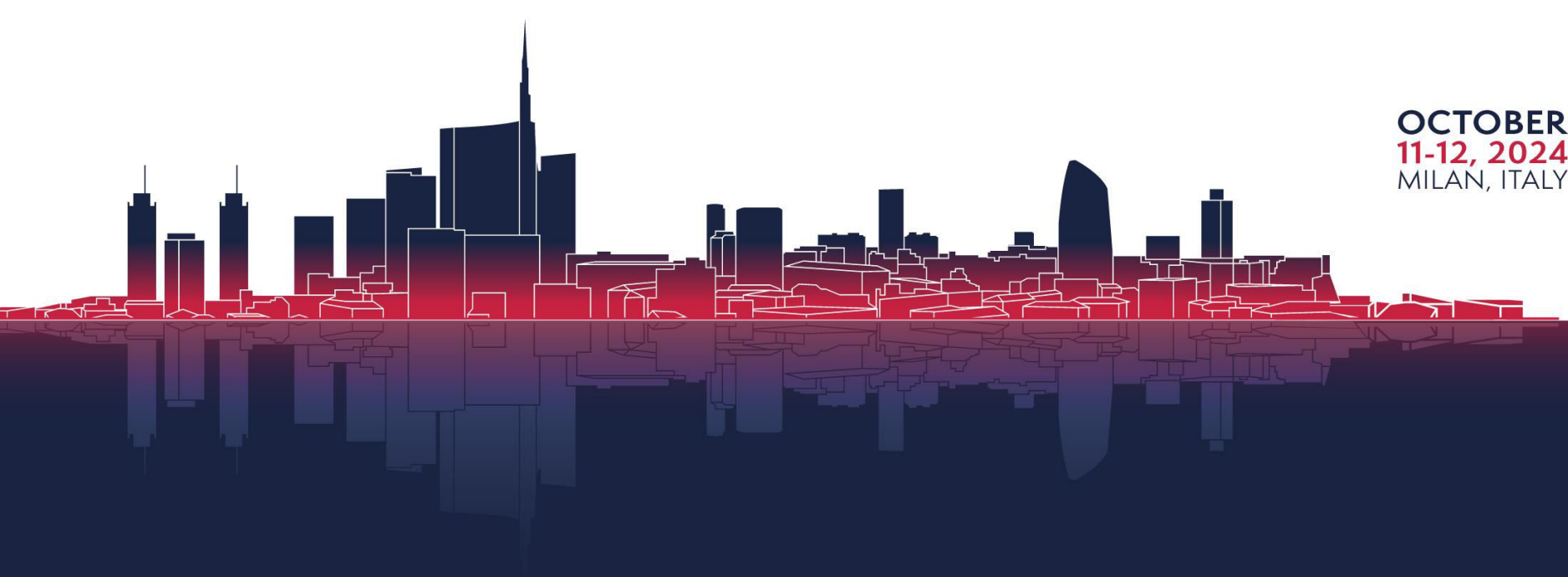

OCTOBER 11-12, 2024 MILAN, ITALY

DeltaCure 3rd International Meeting

Hepatitis delta virus (HDV) replication through HBV integrants in HCC recurrence after liver transplantation

Lorenza Di Marco

DISCLAIMER

- The undersigned declares that there has been no conflict of interest regarding this presentation in the last 24 months

- The presentation does not contain a discussion of investigational or off-label drugs

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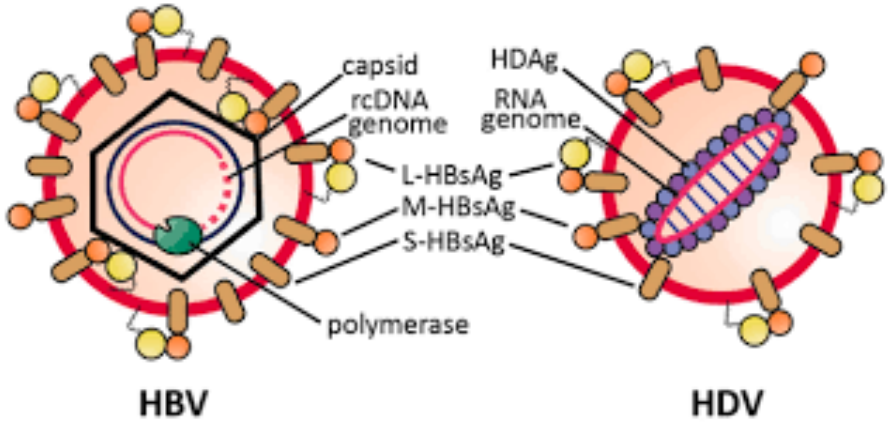
Hepatitis Delta Virus

- Hepatitis D Virus (HDV) is a defective RNA virus requiring the helper function of HBV for viral assembly and in vivo transmission.
- HDV is a highly pathogenic virus that causes the least common but most severe and rapidly progressive chronic hepatitis, leading to cirrhosis in about 80% of the cases within 10 years.
- HDV cirrhosis may be a stable disease for years, BUT a high proportion of patients eventually die of hepatic decompensation or hepatocellular carcinoma (HCC) unless they undergo liver transplantation.



A 52 years-old PWID man with HBV, HDV and HIV infection..

Liver Transplantation for cirrhosis and by HCC

2012

2018

- HBsAg reversion
- Serum HDV-RNA >19,000 IU/mL
- Undetectable HBV-DNA

2019

- Increased transaminase levels
- **HCC recurrence in the left adrenal gland**

↓

Adrenalectomy

2021

CTs: **two HCC nodules in the liver** and **one in the right adrenal gland**

- **TACE** on liver nodules
- **Sorafenib**

HDV-related hepatitis

CTs: increased volume **of right adrenal gland**

2023

- **RFA** on right adrenal gland
- **Bulevirtide**
- **Regorafenib**

Sep 2024

Still alive

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2012 — **Liver transplantation** for cirrhosis and HCC

2018
- HBsAg 267 UI/ml
- Serum HDV-RNA >19,000 IU/mL
- Undetectable HBV-DNA

Increased transaminases.
HCC recurrence in the left adrenal gland
↓
Adrenalectomy

2019

2021

CTs: **two HCC nodules in the liver** and **one in the right adrenal gland**
- **TACE** on liver nodules
- **Sorafenib**

HDV-related hepatitis occurred.
CTs: increased volume **of HCC in right adrenal gland**

2023
- **RFA** on right adrenal gland
- **Bulevirtide**
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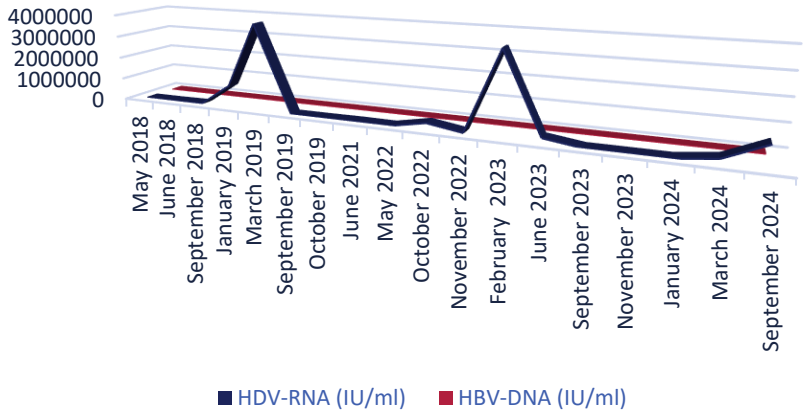
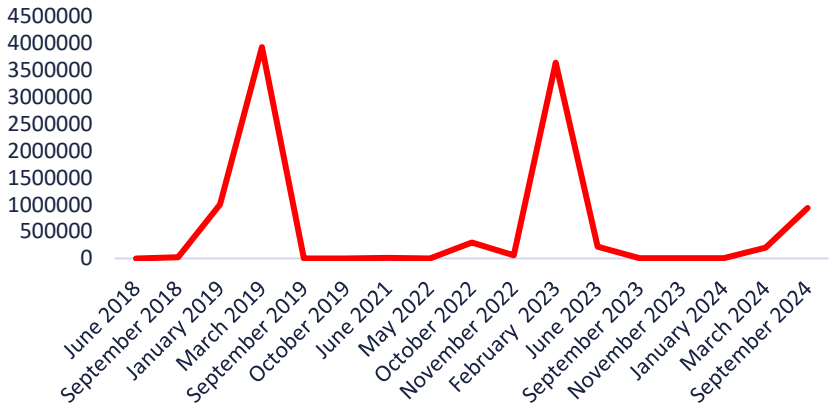
Sep 2024

Still alive

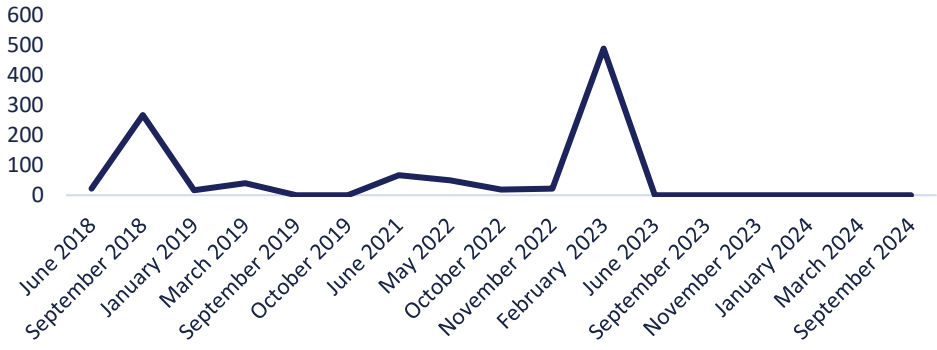
HDV RNA-HBV DNA replication



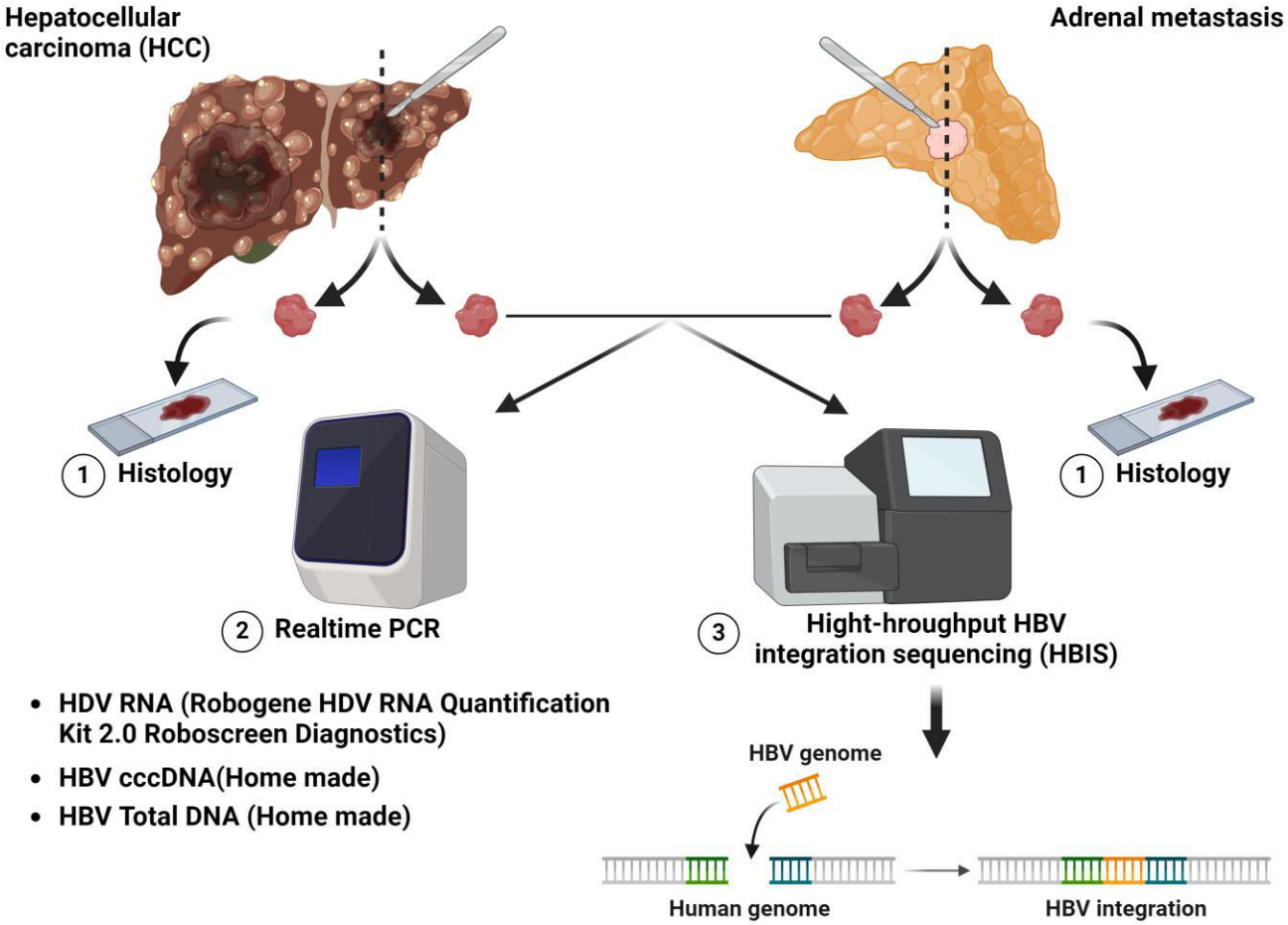
HDV-RNA (IU/ml)



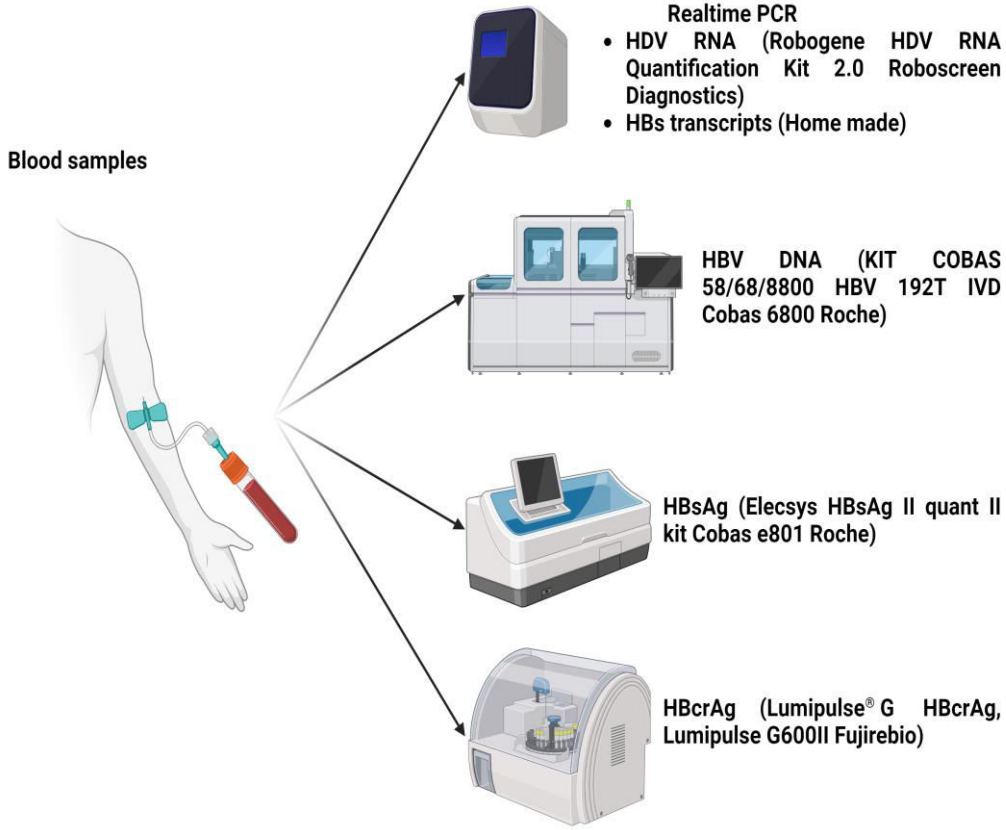
HBsAg (IU/ml)



Materials and Methods



- HDV RNA (Robogene HDV RNA Quantification Kit 2.0 Roboscreen Diagnostics)
- HBV cccDNA(Home made)
- HBV Total DNA (Home made)



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Molecular virology analyses

Analysis of HCC tissue from the explanted liver (2012)

By real-time PCR: HDV-RNA (88,400 copies/cell), HBV-DNA (0.00001 copies/cell), and cccDNA (0.00008 copies/cell)

Analysis of liver biopsy and serum (2019) by real-time PCR

HDV RNA intrahepatic level: 3,920,000 copies/cell

HDV RNA serum level: 214 IU/mL

HBsAg serum level: 60 IU/mL

HBcAg, HBV DNA and HBV cccDNA Intrahepatic levels: undetectable

Analysis of HCC metastasis in the left adrenal gland (2019) by real-time PCR

HDV RNA (5.5 copies/cell), total HBV DNA (0.00001 copies/cells),HBV cccDNA (0.00001 copies/cells)

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HBV integration breakpoints in the human genome

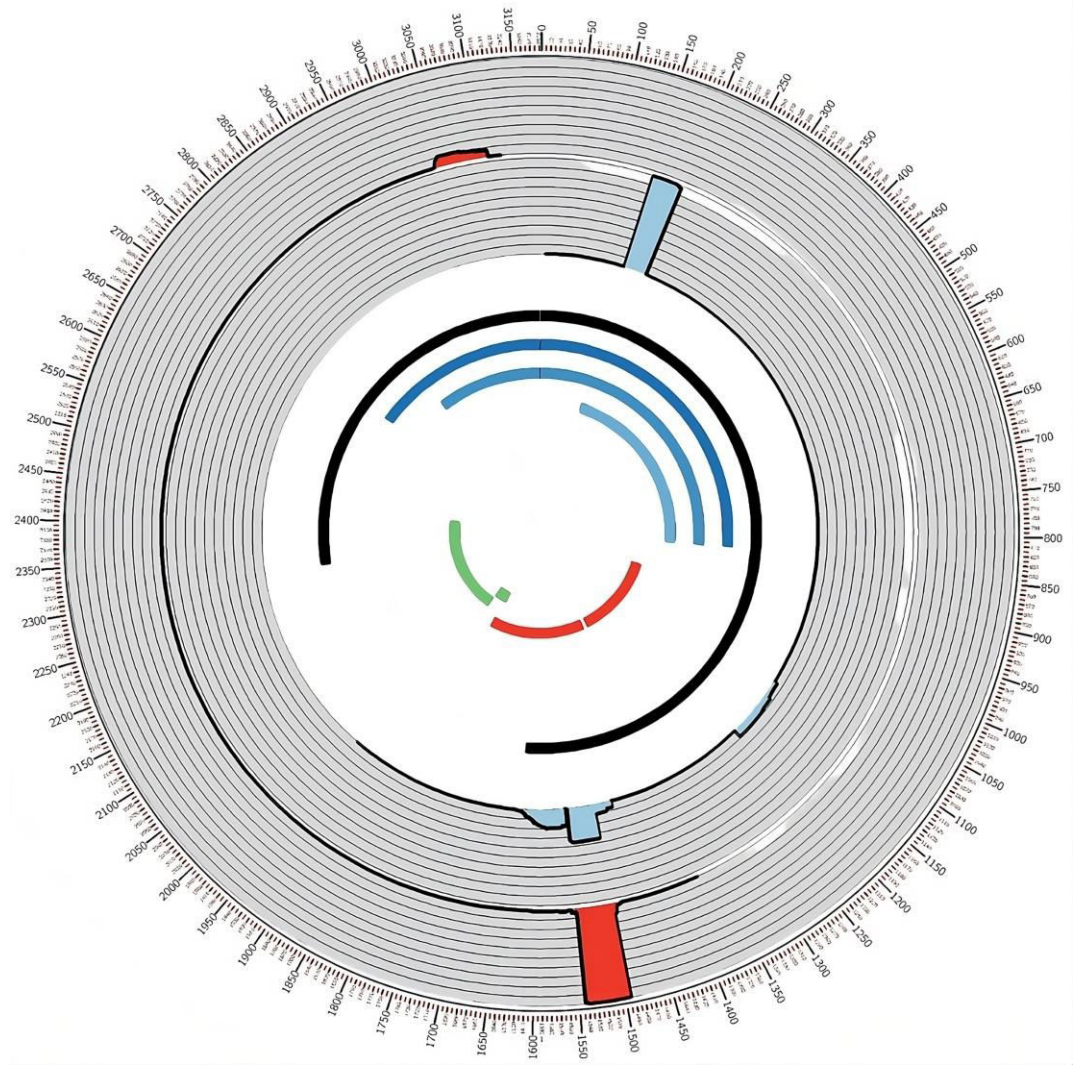
Total number of HBV integration sites	Liver tumor tissue	Adrenal metastasis
8728	1252	7026

The HBV integration sites were also annotated to analyze their distribution in distinct genomic elements

Human genomic elements	Liver tumor tissue %	Adrenal metastasis %
Gene	315/1252 (25.16)	3783/7026 (53.84)
Exon	57/1252 (4.55)	559/7026 (7.96)
CDS	22/1252 (1.76)	200/7026 (2.85)
Intron	264/1252 (21.09)	3253/7026 (46.30)
mRNA	256/1252 (20.45)	2897/7026 (41.23)
lncRNA	83/1252 (6.63)	1048/7026 (14.92)
pseudogene	16/1252 (1.28)	110/7026 (1.57)
Intergenic	922/1252 (73.64)	3134/7026 (44.61)

Frequency of integrations in coding gene regions

Distribution of breakpoints in the HBV genome



Liver tumor tissue

Adrenal metastasis

HBV genomic regions	Liver tumor tissue (%)	Adrenal metastasis (%)
PreS1/PreS2/S	122/1252 (9.74)	4294/7026 (61.12)
X	1131/1252 (90.34)	2723/7026 (38.76)
PreCore/Core	0/1252 (0.00)	10/7026 (0.14)
pol	3/1252 (0.24)	14/7026 (0.20)

Pathways affected by HBV integrations

- 123/8728 (1.4%) HBV integrations were in common between liver tumor tissue and adrenal metastasis
- The main pathways affected by HBV integrations

KEGG pathways	Genes
Cell cycle	• **MCM5,** minichromosome maintenance complex component 5 • **PRKDC,** protein kinase DNA-activated, catalytic subunit • **MAD1L1,** mitotic arrest deficient 1 like 1
Transcriptional misregulation	• **CEBPE,** CCAAT enhancer binding protein epsilon • **RUNX1,** RUNX family transcription factor 1
Insulin signaling pathway	• **FASN,** fatty acid synthase • **RPTOR,** regulatory associated protein of MTOR complex 1
Pathways in cancer	• **COL4A1,** collagen tupe IV alpha 1 chain • **RUNX1,** RUNX family transcription factor 1
MAPK signaling pathway	• **CACNA1C,** calcium voltage-gated channel subunit alpha1 C
ATP-dependent chromatin remodelling	• **EP400,** E1A binding protein p400
TGF-beta signaling pathway	• **THSD4,** thrombospondin type 1 domain containing 4
DNA replication	• **MCM5,** minichromosome maintenance complex component 5

TAKE HOME MESSAGES

This case shows that:

- HDV-RNA may replicate in extrahepatic metastases of HCC, as confirmed by decreasedbio HDV-RNA levels after adrenalectomy and RFA on the right adrenal gland.
- HBV-DNA integration in HCC metastases may lead to the production of HBsAg.
- HBsAg production from integrated HBV-DNA in the absence of HBV replication may result in active HDV-RNA replication.
- The association between RFA and Bulevirtide therapy resulted in a partial virological (HDV-RNA: <2 log) and biochemical response.

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