# Hepatocellular Carcinoma Case Report from the Phase 3 HOPE-B Gene Therapy Trial in Adults with Hemophilia B

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# Disclosures for Steven W. Pipe

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| | |
|---|---|
| **Research Support/P.I.** | **No relevant conflicts of interest to declare** |
| **Employee** | **No relevant conflicts of interest to declare** |
| **Consultant** | **No relevant conflicts of interest to declare** |
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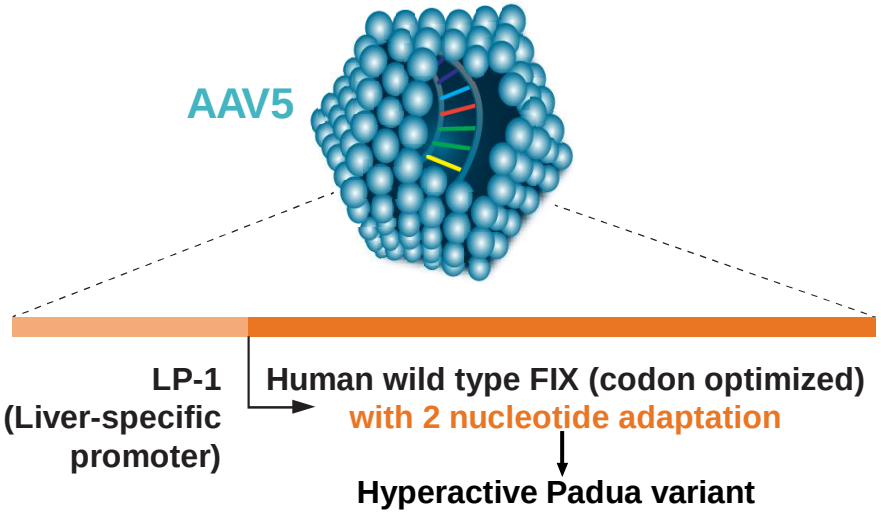
# HOPE-B: Phase 3 AAV5-based Gene Therapy Trial for Hemophilia B

- Open label phase 3 study with follow-up of 54 subjects with hemophilia B receiving a single dose of $2\times10^{13}$ gc/kg of etranacogene dezaparvovec
  - The **largest AAV gene therapy trial cohort in hemophilia B** reported to date
  - Mean FIX activity **significantly increased to near-normal levels** at 6 months post-etranacogene dezaparvovec[1]
  - Most common safety findings at 6 months were transaminase elevations requiring steroid treatment (9 subjects) and infusion-related reactions (7 subjects), supporting **a safety profile consistent with early phase studies**[1,2,3]
- Here we present an SAE of hepatocellular carcinoma (HCC) in a trial subject with multiple pre-existing risk factors for HCC, including the **findings of an independent, expert molecular evaluation** that determined this case was unlikely to be related to treatment with etranacogene dezaparvovec
- Details about the study and **interim safety and efficacy** data **at 52-weeks** after dosing are reported in **PB0653**[4]

**Etranacogene dezaparvovec: Hyperactive FIX Padua variant**





1. Pipe et al; ASH 2020 https://ashpublications.org/blood/article/136/Supplement_2/LBA-6/474189/First-Data-from-the-Phase-3-HOPE-B-Gene-Therapy
2. Leebeek FWG, et al, ASH 2020; Poster #33724
3. Von Drygalski A, et al, ASH 2020; Oral presentation #672.
4. Pipe et al ISTH 2021; 52 Week Efficacy and Safety of Etranacogene Dezaparvovec in Adults with Severe or Moderate-Severe Hemophilia B: Data from the Phase 3 HOPE-B Gene Therapy Trial


AAV, adeno-associated virus; HCC, hepatocellular carcinoma, FIX; factor IX.

# HCC epidemiology and risk factors

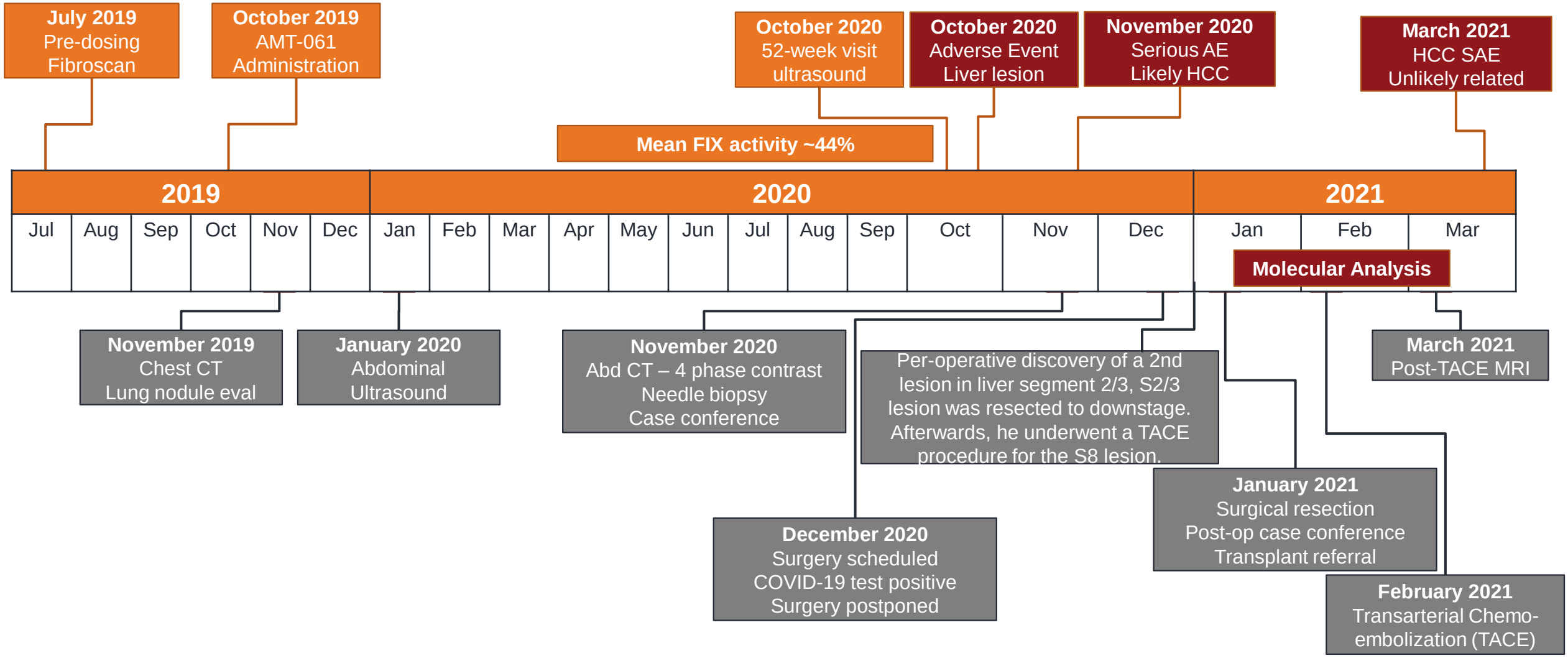
- Primary liver cancer is the sixth most common cancer worldwide.[1]
- Risk factors for development of HCC includes, but not limited to,  Hepatitis C Virus (HCV) and/or Hepatitis B Virus (HBV), advanced age, gender and cirrhosis.[2]
- HCC development has been strongly linked to HBV and HCV infections and is associated with approximately 80% of HCC cases.[3]
- Most cases of HCV-related and HBV-related HCC occur among patients with advanced fibrosis or cirrhosis. However, up to 20% of patients that develop HCC have a non-cirrhotic liver.[2]
- Other risk factors include high alcohol consumption, obesity, exposure to environmental toxins, and metabolic disorders such as NAFLD/NASH.[2,4]
- Although the incidence of HCC is higher in the hemophilic population, it has been correlated with higher incidence of HCV infection and is not due to the hemophilia phenotype.[5]
- Despite clearance of HCV, HCC risk is not eliminated but has been estimated to be reduced by 71%.[6]

1. Sung H et al.,CA Cancer J Clin 2021;71:209–249. 2. Desai A, et al. World J Hepatol. 2019;11(1):1-18. 3. El-Serag HB. Gastroenterology. 2012;142(6):1264-1273. 4. Marrero JA, et al. Hepatology. 2018 Aug;68(2):723-750. doi: 10.1002/hep.29913. 5. Shetty S, et al. Critical Reviews in Oncology/Hematology. 2016;99:129-133. 6.  Ioannou GN, et al. J Hepatol, 5 Sep 2017; doi:10.1016/j.jhep.2017.08.030

# Relevant Medical History

- 69-year-old, white, non-Hispanic male with moderately severe Hemophilia B
  - 1980 – Diagnosed with **HBV** (+ve for anti-HBs, anti-HBc and anti-HBe antibodies)
  - 1983 – Diagnosed with non-A/non-B hepatitis
  - 2003 – Confirmed positive for **HCV** when testing available
  - 2015 – Evaluated for HCV eradication therapy, genotype 1a, no significant fibrosis (Fibroscan 5.7 kPa)
  - 2015 – Treated with paritaprevir/ombitasvir/ritonavir, dasabuvir, and ribavirin; achieving a sustained virologic response
- Social history notable for prior smoking, alcohol consumption of 0-2 units/week
- Familial history notable for cancer

# Timeline of adverse event evaluation/management



# HCC Analysis - Expectations

| | Expected findings if AAV integration drove HCC | Expected findings if HCC independent of AAV treatment |
|---|---|---|
| **Integration Site Analysis** | • Frequent integrations in HCC<br>• Dominant AAV integration site | • Very infrequent integrations<br>• No dominant integration site |
| **Whole Genome Sequencing** | • Integration in/near known oncogenes (eg. TP53, NFE2L2) | • Common HCC oncogene mutations (eg. TP53, NFE2L2)<br>• No AAV integration sites near oncogenes |

# Molecular Analysis: Vector Copy Number and Integration Rate

- Molecular analysis for copy number quantification was conducted via qPCR
  - Vector copy number (VCN) was calculated by normalizing vector copies to the number of housekeeping-gene copies (diploid genomes)

| Tissue | VCN (copies/diploid genome) |
|---|---|
| HCC | 3.21 |
| HCC-adjacent | 4.11 |

- S-EPTS/LM-PCR*, was used to determine the number of integration sites per cell
  - Etranacogene dezaparvovec integration rate into hepatocytes is very low as previously reported for AAV
  - Less than 60 cells out of 250,000 (0.027%) had an integration event in the HCC tumor sample

| Tissue | Integration rate |
|---|---|
| HCC | 0.027% |
| HCC-adjacent | 0.018% |

*Shearing Extension Primer Tag Selection Ligation-Mediated PCR

# Molecular Analysis II: Site of Vector Integration

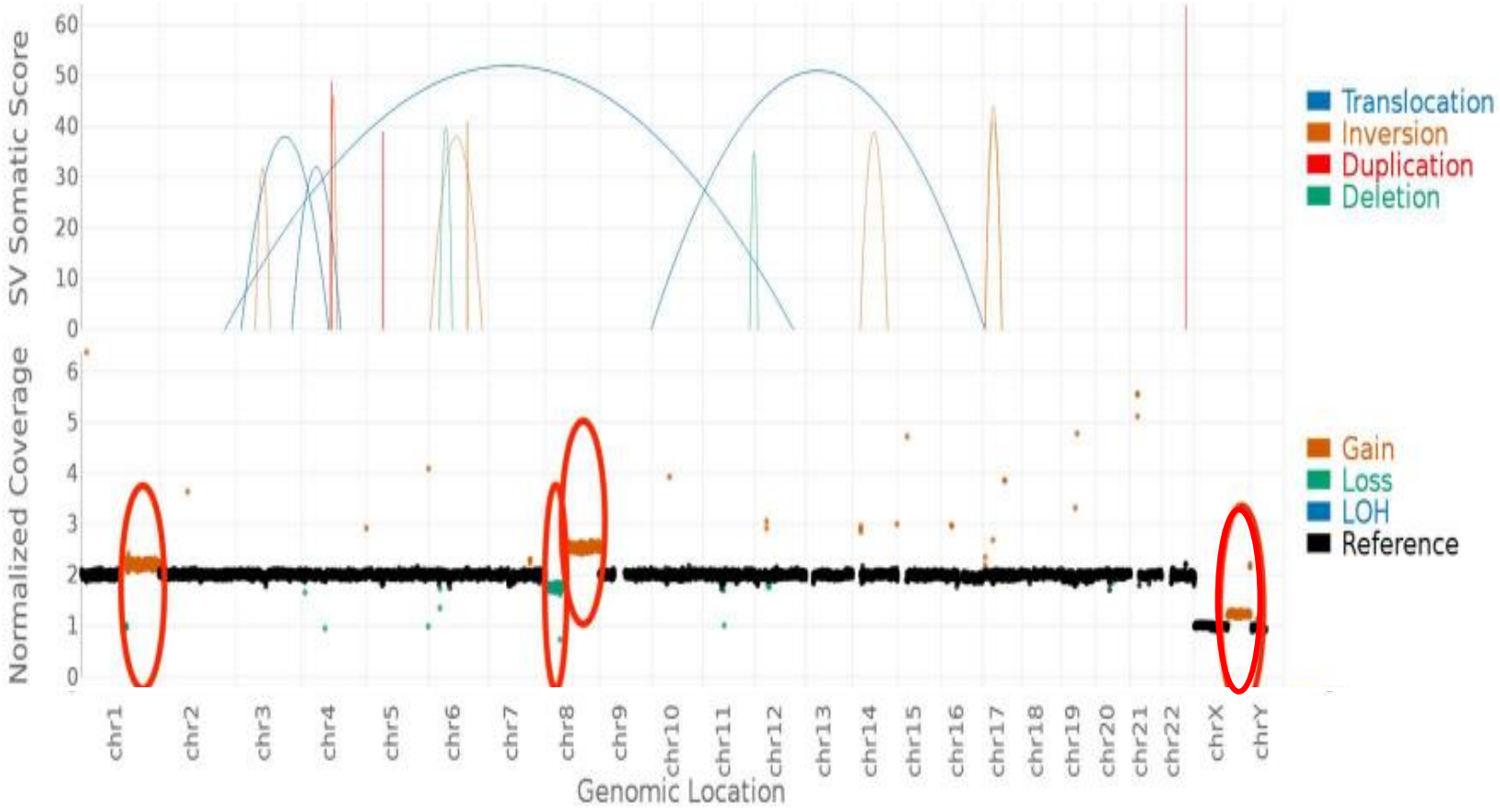
- Integration site (IS) analysis was conducted via whole genome sequencing (WGS)
- No integration event was observed in more than 1 sequence read out of 150 reads
  - The low number of sequence reads for each IS indicate that IS are rare in both the HCC and HCC-adjacent samples.
  - There is no dominant IS in the HCC sample.

| | Chromosome | Integration site | Total sequence count | Gene Name |
|---|---|---|---|---|
| **HCC-adjacent** | Chr19 | 44924479 | 1 | APOC1P1 |
| | Chr5 | 126472870 | 1 | GRAMD3 |

| | Chromosome | Integration site | Total sequence count | Gene Name |
|---|---|---|---|---|
| **HCC** | Chr19 | 44924545 | 1 | APOC1P1 |
| | Chr5 | 54714322 | 1 | EML6 |
| | Chr4 | 91418750 | 1 | CCSER1 |

# Molecular Analysis III: genome-wide chromosomal rearrangements

Whole Genome Sequence analysis




- Multiple structural variants were observed in HCC sample, including mutations in TP53 and NFE2L2 - known to be common drivers of HCC - independent of etranacogene dezaparvovec
- Large chromosomal rearrangements in Chr 1,8 and X characteristic of HCC - independent of etranacogene dezaparvovec

# HCC Analysis: Summary of Results

| | Expected findings if AAV integration drove HCC | Expected findings if HCC independent of AAV treatment | Actual findings of GeneWerk analysis |
|---|---|---|---|
| **Integration Site Analysis** | • Frequent integrations in HCC<br>• Dominant AAV integration site | • Very infrequent integrations<br>• No dominant integration site | • Etranacogene dezaparvovec integration rate into hepatocytes is very low as previously reported for AAV<br>• No dominant integration event or integration site in the HCC sample<br>• Less than 60 cells out of 250K (0.027%) had an integration event in the tumor sample |
| **Whole Genome Sequencing** | • Integration in/near known oncogenes (eg. TP53, NFE2L2) | • Common HCC oncogene mutations<br>• No AAV integration sites near oncogenes | • Very low rate of vector integration in genes not known to be associated with HCC<br>• Large chromosomal rearrangements in Chr 1,8 and X characteristic of HCC - independent of etranacogene dezaparvovec<br>• Mutations in TP53 and NFE2L2 - known to be common drivers of HCC - independent of etranacogene dezaparvovec |

# Conclusions and Future Recommendations

- Asymptomatic HCC was identified in an older subject with HBV, prior HCV post SVR on a routine safety ultrasound 1 year after dosing; the subject has been treated with TACE and is under evaluation for liver transplant
- HCC development in this case is now considered unlikely related to treatment with etranacogene dezaparvovec based upon the results of genetic analysis and pre-existing risk factors
- Short-term and long-term follow-up is important after gene therapy
  - Many patients with hemophilia have pre-existing risk factors for HCC
  - The risk of HCC after HCV-SVR is still being investigated
  - Aging patients may develop risk factors over time unrelated to treatment (age >50, NAFLD/NASH, obesity, alcohol use)
- Ultrasound monitoring of all participants enrolled in etranacogene dezaparvovec clinical trials was increased to twice yearly regardless of pre-existing risk factors for HCC as a conservative approach.