

## **Cellular, Tissue, and Gene Therapies Advisory Committee September 2-3, 2021 Meeting Presentation**

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# rAAV Integration:

## *In Vitro* & Mice

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# Disclosures

Co-Founder – JAYA Biosciences

Consultant – BioMarin Pharmaceutical

Consultant – Pfizer

Consultant – Taysha Gene Therapies

Scientific Advisory Board – M6P Therapeutics

# rAAV Integration:

## *In Vitro* & Mice

### Chronological:

- A) 1997-2001 – Prior to the observation of hepatocellular carcinoma (HCC)
- B) 2001-2016 – rAAV integration and associated HCC in mice
- C) Conditions affecting rAAV-associated HCC

# rAAV Integration

(1997-2001)

JOURNAL OF VIROLOGY, Nov. 1997, p. 8429–8436  
0022-538X/97/\$04.00+0  
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Vol. 71, No. 11

## Adeno-Associated Virus Vector Integration Junctions

ELIZABETH A. RUTLEDGE AND DAVID W. RUSSELL\*

- 1) *In vitro* (HeLa cells) integration analysis
- 2) Random integration
- 3) None of the integrated vectors were fully intact

# rAAV Integration

(1997-2001)

JOURNAL OF VIROLOGY, Dec. 1997, p. 9231-9247  
0022-538X/97/\$04.00+0  
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Vol. 71, No. 12

## Cellular Recombination Pathways and Viral Terminal Repeat Hairpin Structures Are Sufficient for Adeno-Associated Virus Integration In Vivo and In Vitro

C. C. YANG,<sup>1</sup> X. XIAO,<sup>1</sup> X. ZHU,<sup>1†</sup> D. C. ANSARDI,<sup>1‡</sup> N. D. EPSTEIN,<sup>2</sup> M. R. FREY,<sup>3</sup>  
A. G. MATERA,<sup>3</sup> AND R. J. SAMULSKI<sup>1\*</sup>

- 1) Integration analysis in cell lines and a cell-free system
- 2) No obvious site preference for integration.
- 3) No intact ITRs were identified
- 4) Favor actively transcribed regions

# rAAV Integration

(1997-2001)

JOURNAL OF VIROLOGY, July 1999, p. 5438–5447

Vol. 73, No. 7

0022-538X/99/\$04.00+0

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## Isolation of Recombinant Adeno-Associated Virus Vector-Cellular DNA Junctions from Mouse Liver

HIROYUKI NAKAI,<sup>1,2,3\*</sup> YUICHI IWAKI,<sup>2</sup> MARK A. KAY,<sup>3</sup> AND LINDA B. COUTO<sup>1</sup>

- 1) *In vivo* integration analysis (mouse liver)
- 2) Integrated vectors were rearranged (ITRs and vector sequences)
- 3) Two integrants were identified in genes ( $\alpha 1$  collagen, rRNA)

# rAAV Integration

(1997-2001)

JOURNAL OF VIROLOGY, Aug. 2001, p. 6969–6976  
0022-538X/01/\$04.00+0 DOI: 10.1128/JVI.75.15.6969–6976.2001  
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Vol. 75, No. 15

## Extrachromosomal Recombinant Adeno-Associated Virus Vector Genomes Are Primarily Responsible for Stable Liver Transduction In Vivo

HIROYUKI NAKAI, STEPHEN R. YANT, THERESA A. STORM, SALLY FUESS,  
LEONARD MEUSE, AND MARK A. KAY\*

- 1) Extrachromosomal rAAV is the primary source of expression
- 2) Only 5-10% of rAAV vector are integrated into the host genome
- 3) Low level integration, increased safety profile



# Summary 1

(1997-2001)

- 1) Relatively low level of integration
- 2) Chromosome/vector junctions are within or near ITRs
- 3) ITRs are rearranged
- 4) No or minimal homology between cellular and vector sequences
- 5) No integration 'hot spots' were identified
- 6) Integration mechanism is unknown
- 7) No toxicity observed up to this point

# rAAV Integration

(2001-2016)

Gene Therapy (2001) 8, 1343–1346

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[www.nature.com/gt](http://www.nature.com/gt)

## **BRIEF COMMUNICATION**

### *Observed incidence of tumorigenesis in long-term rodent studies of rAAV vectors*

A Donsante<sup>1</sup>, C Vogler<sup>2</sup>, N Muzyczka<sup>3</sup>, JM Crawford<sup>4</sup>, J Barker<sup>5</sup>, T Flotte<sup>3</sup>,  
M Campbell-Thompson<sup>4</sup>, T Daly<sup>1,6</sup> and MS Sands<sup>1</sup>

- 1) Long-term (18mo) study in MPSVII mouse
- 2) IV administration at birth ( $\sim 1 \times 10^{14}$  vg/kg)
- 3) Persistent expression, dramatic clinical/behavioral improvements
- 4) 3/5 rAAV-treated animals had HCC at 18 months of age
- 5) Impossible to determine if rAAV caused HCC

# rAAV Integration

(2001-2016)

Adeno-associated virus vectors integrate at chromosome breakage sites

Daniel G Miller<sup>1</sup>, Lisa M Petek<sup>2</sup> & David W Russell<sup>2,3</sup>

NATURE GENETICS VOLUME 36 | NUMBER 7 | JULY 2004

- 1) rAAV does not increase mutation rate
- 2) rAAV integrates at spontaneous or induced double strand breaks.

# rAAV Integration

(2001-2016)

JOURNAL OF VIROLOGY, Mar. 2005, p. 3606–3614  
0022-538X/05/\$08.00+0 doi:10.1128/JVI.79.6.3606–3614.2005  
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Vol. 79, No. 6

## Large-Scale Molecular Characterization of Adeno-Associated Virus Vector Integration in Mouse Liver

Hiroyuki Nakai,<sup>1\*</sup> Xiaolin Wu,<sup>2</sup> Sally Fuess,<sup>1</sup> Theresa A. Storm,<sup>1,3</sup> David Munroe,<sup>2</sup> Eugenio Montini,<sup>4,†</sup>  
Shawn M. Burgess,<sup>5</sup> Markus Grompe,<sup>4,6</sup> and Mark A. Kay<sup>1,3</sup>

- 1) Analyzed 347 rAAV integration sites in mouse liver
- 2) Integration ‘hot spot’ was found in rRNA gene repeats
- 3) >50% of integrations occurred near transcription start sites or CpG islands



# rAAV Integration

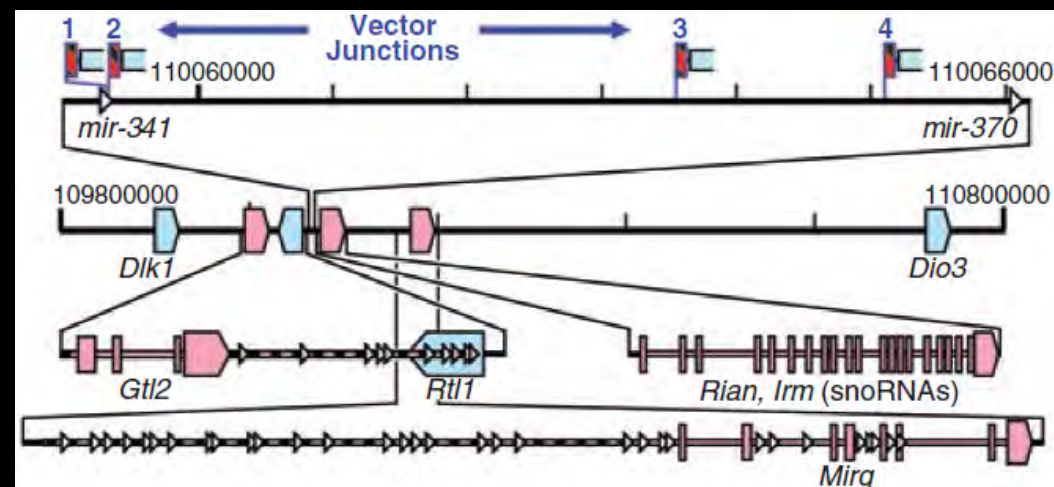
(2001-2016)

## AAV Vector Integration Sites in Mouse Hepatocellular Carcinoma

Anthony Donsante,<sup>1\*</sup> Daniel G. Miller,<sup>2\*</sup> Yi Li,<sup>3,4</sup> Carole Vogler,<sup>5</sup> Elizabeth M. Brunt,<sup>5</sup>  
David W. Russell,<sup>3,4†</sup> Mark S. Sands<sup>1,6†</sup>

SCIENCE VOL 317 27 JULY 2007

- 1) 15/34 rAAV-treated mice developed HCC (6/18 MPSVII, 9/16 WT)
- 2) Integrated rAAV sequences were isolated from HCC samples from 4 mice
- 3) In each case, rAAV integrated in the mouse *Rian* locus
- 4) Several downstream genes and  $\mu$ RNAs were dysregulated



# rAAV Integration

(2001-2016)

## Long-Term Correction of Sandhoff Disease Following Intravenous Delivery of rAAV9 to Mouse Neonates

Jagdeep S Walia<sup>1,2,3,4</sup>, Naderah Altaieb<sup>2</sup>, Alexander Bello<sup>5,6</sup>, Christa Kruck<sup>2</sup>, Matthew C LaFave<sup>7</sup>, Gaurav K Varshney<sup>7</sup>, Shawn M Burgess<sup>7</sup>, Biswajit Chowdhury<sup>2</sup>, David Hurlbut<sup>8</sup>, Richard Hemming<sup>2</sup>, Gary P Kobinger<sup>5,6</sup> and Barbara Triggs-Raine<sup>2,3,4</sup>

- 1) 8/10 rAAV-treated Sandhoff mice developed HCC
- 2) IV injection of rAAV in neonatal mice ( $2.5 \times 10^{14}$  vg/kg)
- 3) Several tumors had rAAV integrations in the *Rian* locus

# rAAV Integration

(2001-2016)

## Vector design influences hepatic genotoxicity after adeno-associated virus gene therapy

Randy J. Chandler,<sup>1</sup> Matthew C. LaFave,<sup>2</sup> Gaurav K. Varshney,<sup>2</sup> Niraj S. Trivedi,<sup>3</sup> Nuria Carrillo-Carrasco,<sup>4</sup> Julien S. Senac,<sup>1</sup> Weiwei Wu,<sup>5</sup> Victoria Hoffmann,<sup>6</sup> Abdel G. Elkahloun,<sup>5</sup> Shawn M. Burgess,<sup>2</sup> and Charles P. Venditti<sup>1</sup>

J Clin Invest (2015) 125:870-880

- 1) 64/95 mice treated with rAAV ( $1 \times 10^{14}$  vg/kg) at birth developed HCC
- 2) Confirmed the *Rian* locus as a 'hot spot' for rAAV integration
- 3) HCC appears to be dose-dependent
- 4) Strong promoter/enhancer combinations increased incidence of HCC
- 5) HCC independent of transgene
- 6) HCC independent of genotype



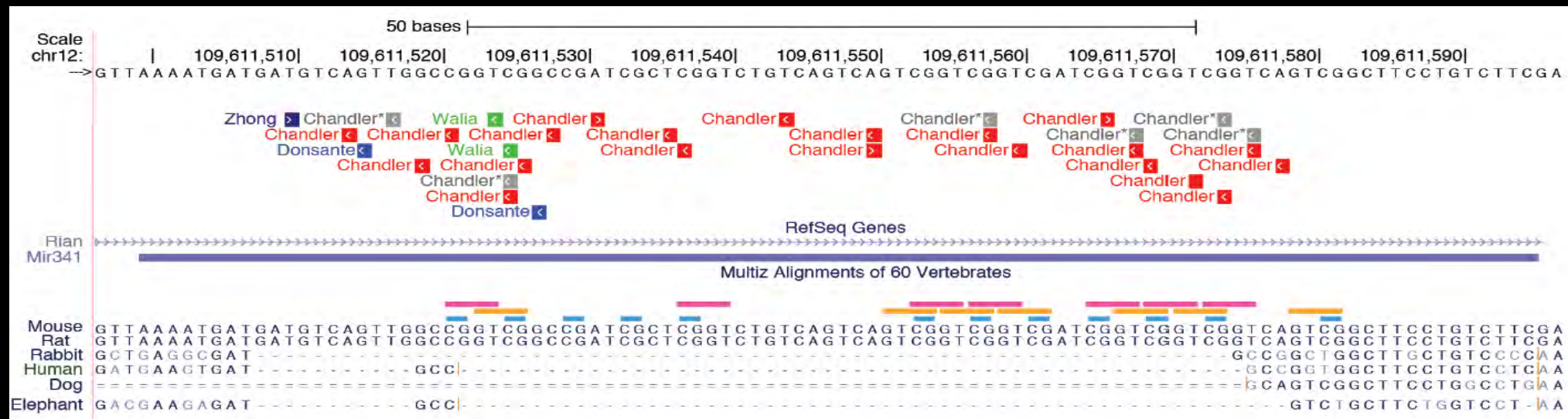
# rAAV Integration

## (2001-2016)

# Genotoxicity in Mice Following AAV Gene Delivery: A Safety Concern for Human Gene Therapy?

Randy J Chandler<sup>1</sup>, Matthew C LaFave<sup>2</sup>, Gaurav K Varshney<sup>2</sup>, Shawn M Burgess<sup>2</sup> and Charles P Venditti<sup>1</sup>

Molecular Therapy vol. 24 no. 2 february 2016



- 1) Compilation of rAAV integration sites across several studies
- 2) Many *Rian* integrations occur in a region (~60bp) unique to rodents



# Summary 2

(2001-2016)

- 1) Systemic delivery of a rAAV vector can cause HCC in mice
- 2) rAAV integration into the mouse *Rian* locus is associated with HCC
- 3) Many rAAV integrants are located in *Rian* sequences unique to rodents
- 4) Highest frequency of HCC if administered during the newborn period
- 5) Strong promoter/enhancer combinations increase frequency
- 6) rAAV-associated HCC appears to be dose-dependent
- 7) Low frequency if administered in adult animals
- 8) No HCC following CNS-directed rAAV-mediated gene therapy

# rAAV Integration

(recent findings)

## Liver Injury Increases the Incidence of HCC following AAV Gene Therapy in Mice

Dhwanil A. Dalwadi,<sup>1,2</sup> Laura Torrens,<sup>3</sup> Jordi Abril-Fornaguera,<sup>3</sup> Roser Pinyol,<sup>3</sup> Catherine Willoughby,<sup>3</sup> Jeffrey Posey,<sup>2</sup> Josep M. Llovet,<sup>3,4,5</sup> Christian Lanciault,<sup>6</sup> David W. Russell,<sup>7,8</sup> Markus Grompe,<sup>2</sup> and Willscott E. Naugler<sup>1</sup>

Molecular Therapy Vol. 29 No 2 February 2021

- 1) Increased incidence of HCC in adult mice with non-alcoholic fatty liver
- 2) *Rian*-targeted construct – 100% penetrance
- 3) Non-targeted construct – 50% penetrance

# rAAV Integration

(recent findings)

## Enhanced Efficacy and Increased Long-Term Toxicity of CNS-Directed, AAV-Based Combination Therapy for Krabbe Disease

Yedda Li,<sup>1</sup> Christopher A. Miller,<sup>1</sup> Lauren K. Shea,<sup>1</sup> Xuntian Jiang,<sup>1</sup> Miguel A. Guzman,<sup>2</sup> Randy J. Chandler,<sup>3</sup> Sai M. Ramakrishnan,<sup>1</sup> Stephanie N. Smith,<sup>3</sup> Charles P. Venditti,<sup>3</sup> Carole A. Vogler,<sup>2</sup> Daniel S. Ory,<sup>1</sup> Timothy J. Ley,<sup>1,4</sup> and Mark S. Sands<sup>1,4</sup>

Molecular Therapy Vol. 29 No 2 February 2021 ©

- 1) HCC development following CNS-directed rAAV-mediated gene therapy
- 2) Combination therapy (HSCT transplantation + small molecule drug)
- 3) ~95% penetrance in combination-treated Krabbe & WT mice

# Targeted Sequence Capture

Animal ID	Age <sup>a</sup> (months)	Chr	Integration <sup>b</sup> Start Site (bp)	Read Counts	Gene	Gene Description	Reference
6657	13.4	12	109643597 <sup>c</sup>	857	<i>Rian</i>	microRNA cluster	33
6675	14.7	12	109618074 <sup>c</sup>	1,390	<i>Rian</i>	microRNA cluster	33
		6	99150006 <sup>c</sup>	4,883	<i>Foxp1</i>	Forkhead protein, tumor suppressor	28,29
		8	39104763 <sup>c</sup>	199	<i>Tusc3</i>	endoplasmic reticulum (ER) protein, candidate tumor suppressor	30
		12	109631801 <sup>c</sup>	3,060	<i>Rian</i>	microRNA cluster	33
6722	17.6	7	75627402 <sup>c</sup>	1,682	<i>Akap13</i>	A-kinase anchor protein, double oncogene homology, breast cancer	25,34
6815	14.7	12	109609803 <sup>c</sup>	1,684	<i>Rian</i>	microRNA cluster	33
6824	15.0	12	109631309 <sup>c</sup>	213	<i>Rian</i>	microRNA cluster	33
		1	192215900 <sup>c</sup>	55	<i>Kcnh1</i>	K <sup>+</sup> channel, increased expression confers growth advantage	26,35
		6	81973046 <sup>d</sup>	87	<i>Eva1a</i>	regulator of programmed cell death	27,36
6828	13.1	12	109625075 <sup>c</sup>	3,737	<i>Rian</i>	microRNA cluster	33
6902	14.9	12	109613953 <sup>c</sup>	85	<i>Rian</i>	microRNA cluster	33
		12	109671965 <sup>c</sup>	825	<i>Rian</i>	microRNA cluster	33
		6	94142817 <sup>c</sup>	65	<i>Magil</i>	membrane-associated guanylate kinase, candidate tumor suppressor	31,32
7025	15.5	12	109615046	1,430	<i>Rian</i>	microRNA cluster	33
7045	16.8	12	109606198 <sup>c</sup>	3,519	<i>Rian</i>	microRNA cluster	33
7046	14.2	12	109611352 <sup>c</sup>	387	<i>Rian</i>	microRNA cluster	33

- 1) All tumors had rAAV integrations in the *Rian* locus
- 2) 4/10 tumors had rAAV integrations in candidate tumor suppressors, cancer-associated genes, or genes that regulate cell growth or death



# Conclusions

- 1) rAAV vectors can stably integrate into the mouse genome ( $\leq 10\%$ )
- 2) The vast majority of integrated vectors appear to be grossly rearranged
- 3) The integration mechanism is unknown
- 4) Integration into the mouse *Rian* locus is associated with HCC
- 5) HCC formation appears to be dose dependent
- 6) Strong promoter/enhancer combinations increase HCC frequency
- 7) Low frequency of HCC in adult animals
- 8) Low frequency of HCC following CNS-directed gene therapy
- 9) Liver injury (eg. NAFL) can exacerbate HCC phenotype
- 10) Adjunct therapies with mild oncogenic potential exacerbate HCC phenotype

# Acknowledgements

## Washington University

Anthony Donsante  
Yedda Li  
Christopher Miller  
Timothy Ley

## University of Washington

Daniel Miller  
David Russell

## NIH

Randy Chandler  
Charles Venditti

# Additional Information

Recent round table discussion on rAAV integration

Sponsored by the American Society of Gene and Cell Therapy (ASGCT)

August 18, 2021

[https://www.youtube.com/watch?v=L\\_4luK3fNU0](https://www.youtube.com/watch?v=L_4luK3fNU0)