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 Vol. 71, No. 11
0022-538X/97/$04.00+0

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

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,[1] X. XIAO,[1] X. ZHU,[1]† D. C. ANSARDI,[1]‡ N. D. EPSTEIN,[2] M. R. FREY,[3] A. G. MATERA,[3] AND R. J. SAMULSKI[1]*

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

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

HIROYUKI NAKAI,[1,2,3]* YUICHI IWAKI,[2] MARK A. KAY,[3] AND LINDA B. COUTO[1]

1) *In vivo* integration analysis (mouse liver)
2) Integrated vectors were rearranged (ITRs and vector sequences)
3) Two integrants were identified in genes (α1 collagen, rRNA)

rAAV Integration

(1997-2001)

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

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

www.nature.com/gt

BRIEF COMMUNICATION

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

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

1) Long-term (18mo) study in MPSVII mouse
2) IV administration at birth (~$1x10^{14}$ 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[1], Lisa M Petek[2] & David W Russell[2,3]

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 Vol. 79, No. 6
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.6.3606–3614.2005

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

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

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

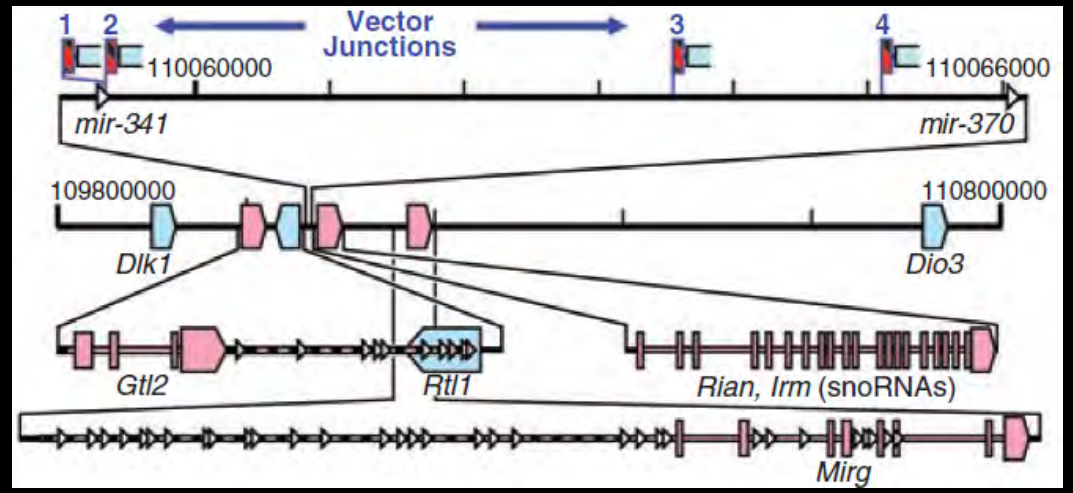
(2001-2016)

AAV Vector Integration Sites in Mouse Hepatocellular Carcinoma

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

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 μRNAs were dysregulated



rAAV Integration

(2001-2016)

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

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

1) 8/10 rAAV-treated Sandhoff mice developed HCC
2) IV injection of rAAV in neonatal mice (2.5×10^{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,[1] Matthew C. LaFave,[2] Gaurav K. Varshney,[2] Niraj S. Trivedi,[3] Nuria Carrillo-Carrasco,[4] Julien S. Senac,[1] Weiwei Wu,[5] Victoria Hoffmann,[6] Abdel G. Elkahloun,[5] Shawn M. Burgess,[2] and Charles P. Venditti[1]

J Clin Invest (2015) 125:870-880

1) 64/95 mice treated with rAAV ($1x10^{14}$ 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

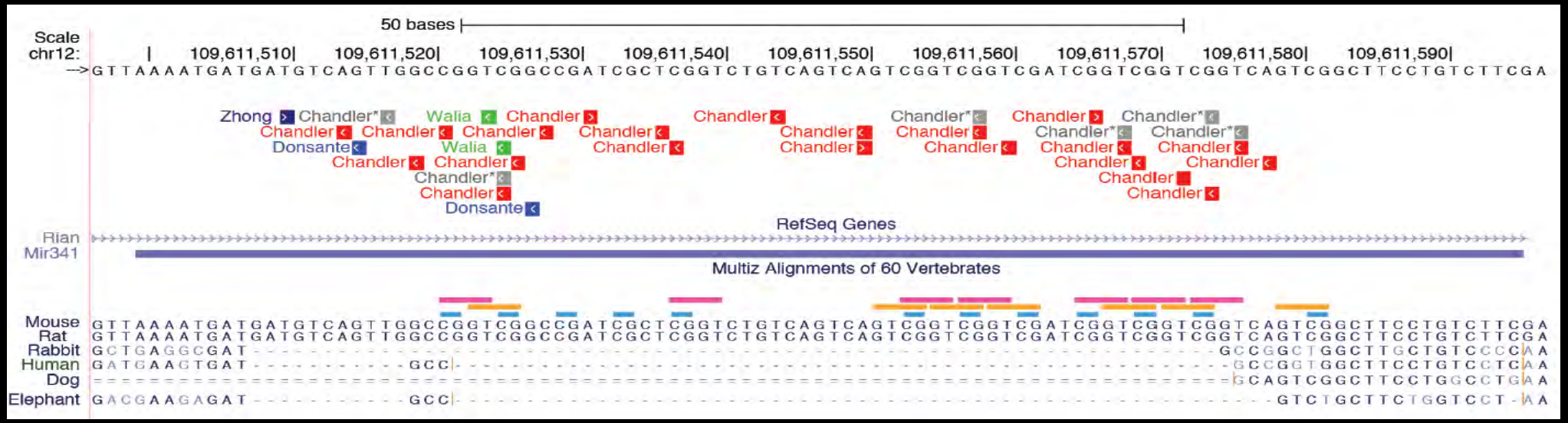
rAAV Integration

(2001-2016)

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

Randy J Chandler[1], Matthew C LaFave[2], Gaurav K Varshney[2], Shawn M Burgess[2] and Charles P Venditti[1]

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

Molecular Therapy Vol. 29 No 2 February 2021

1) Increased incidence of HCC in <u>adult</u> 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,[1] Christopher A. Miller,[1] Lauren K. Shea,[1] Xuntian Jiang,[1] Miguel A. Guzman,[2] Randy J. Chandler,[3] Sai M. Ramakrishnan,[1] Stephanie N. Smith,[3] Charles P. Venditti,[3] Carole A. Vogler,[2] Daniel S. Ory,[1] Timothy J. Ley,[1,4] and Mark S. Sands[1,4]

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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

Table 1. AAV Integration Sites in or near Cancer- and Cell-Growth/Death-Associated Genes

Animal ID	Age[a] (months)	Chr	Integration[b] Start Site (bp)	Read Counts	Gene	Gene Description	Reference
6657	13.4	12	109643597[c]	857	*Rian*	microRNA cluster	33
6675	14.7	12	109618074[c]	1,390	*Rian*	microRNA cluster	33
		6	99150006[c]	4,883	*Foxp1*	Forkhead protein, tumor suppressor	28,29
		8	39104763[c]	199	*Tusc3*	endoplasmic reticulum (ER) protein, candidate tumor suppressor	30
6722	17.6	12	109631801[c]	3,060	*Rian*	microRNA cluster	33
		7	75627402[c]	1,682	*Akap13*	A-kinase anchor protein, double oncogene homology, breast cancer	25,34
6815	14.7	12	109609803[c]	1,684	*Rian*	microRNA cluster	33
6824	15.0	12	109631309[c]	213	*Rian*	microRNA cluster	33
		1	192215900[c]	55	*Kcnh1*	K^+ channel, increased expression confers growth advantage	26,35
		6	81973046[d]	87	*Eva1a*	regulator of programmed cell death	27,36
6828	13.1	12	109625075[c]	3,737	*Rian*	microRNA cluster	33
6902	14.9	12	109613953[c]	85	*Rian*	microRNA cluster	33
		12	109671965[e]	825	*Rian*	microRNA cluster	33
		6	94142817[c]	65	*Magi1*	membrane-associated guanylate kinase, candidate tumor suppressor	31,32
7025	15.5	12	109615046	1,430	*Rian*	microRNA cluster	33
7045	16.8	12	109606198[c]	3,519	*Rian*	microRNA cluster	33
7046	14.2	12	109611352[c]	387	*Rian*	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 (≤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

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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