Shedding after *in situ* rAAV delivery

Review of the literature and 8 years of experience in Nantes in large animal models

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Mode of delivery	Animal Models in Nantes	rAAV serotype	Maximal time course
Intracerebral	Spinal Muscular Atrophy Cat Mucopolysaccharidosis Type I Dog Nonhuman primate	1, 2, 5	8 months
Intrathecal	Spinal Muscular Atrophy Cat	1	3 months
Subretinal	RPE65 deficient Dog Normal dog Nonhuman primate	2, 4, 5	6 years
Intravitreal	Normal dog	2	4 months
Isolated limb infusion	Nonhuman primate	1, 8	2 years
Intramuscular	Nonhuman primate	1, 2, 8	5 years
Intrathymic	Nonhuman primate	8	1 month

Mode of delivery:

Intracerebral

Animal Model:

Cat, Dog, Primate

rAAV serotype:

1, 2, 5

rAAV dose (total):

$1{,}4 \times 10^{10}$vg to $4{,}6 \times 10^{11}$vg

Ciron *et al.*,
Annals of Neurol., 2006
Ciron *et al.*,
Manuscript in preparation
Joussemet *et al.*,
Manuscript in preparation

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	>100 vg/cell (≥ month 8)	+ (≥ month 6)	+ (≥ month 8)
Serum	+ (up to day 4)	+ (up to day 10)	nd	nd
Cerebrospinal Fluid	-	+ (≥ month 3)	nd	nd
Urine	nd	nd	nd	nd
PBMC	N/A	+ (up to month 2)	nd	nd
Lymph nodes	N/A	+ (≥ month 3)	nd	nd
Spleen	N/A	pending	nd	nd
Liver	N/A	+ (≥ month 3)	nd	nd
Gonads	N/A	-	nd	nd

Humoral response	α-transgene	+
	α-capsid	+
Cellular response	α-transgene	+ ?
	α-capsid	+ ?

nd = not done

Mode of delivery:

Intrathecal

Animal Model:

Cat

rAAV serotype:

1

rAAV dose (total):

$4{,}6 \times 10^{11}$vg to $1{,}4 \times 10^{12}$vg

Joussemet *et al.*,
Manuscript in preparation

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	+ (≥ month 3)	nd	+ (≥ month 3)
Serum	nd	+ (up to month 2)	nd	nd
Cerebrospinal Fluid	+ (up to day 3)	+ (up to month 3)	nd	nd
Urine	nd	nd	nd	nd
PBMC	N/A	+ (up to month 2)	nd	nd
Lymph nodes	N/A	pending	nd	nd
Spleen	N/A	pending	nd	nd
Liver	N/A	pending	nd	nd
Gonads	N/A	pending	nd	nd

Humoral response	α-transgene	nd
	α-capsid	nd
Cellular response	α-transgene	nd
	α-capsid	nd

nd = not done

Mode of delivery:

Subretinal/Intravitreal

Animal Model:

Dog, Primate

rAAV serotype:

2, 4, 5

rAAV dose (total):

$1{,}6 \times 10^{10}$vg to 5×10^{11}vg

Provost *et al.*,
Mol. Ther., 2005 + unpub. data
Stieger *et al.*,
Mol. Ther., 2006
Le Meur *et al.*,
Gene Ther., 2006

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	44 vg/cell (≥ year 1,5)	nd	+ (≥ year 6)
Serum	nd	+ (up to day 14)	nd	nd
Cerebrospinal Fluid	nd	-	nd	nd
Urine	nd	nd	nd	nd
PBMC	N/A	+ (≥ month 3)	nd	nd
Lymph nodes	N/A	+ (≥ month 3)	nd	nd
Spleen	N/A	nd	nd	nd
Liver	N/A	-	nd	nd
Gonads	N/A	-	nd	nd

Humoral response	α-transgene	+
	α-capsid	-
Cellular response	α-transgene	nd
	α-capsid	nd

nd = not done

Mode of delivery:

Isolated limb infusion

Animal Model:

Primate

rAAV serotype:

1, 8

rAAV dose (total):

$6{,}7 \times 10^{12}$vg to $1{,}2 \times 10^{13}$vg

Toromanoff *et al.*,
Manuscript in preparation

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	1 to 20 vg/cell (≥ year 2)	+ (≥ year 2)	+ (≥ year 2)
Serum	+ (up to day 7)	+ (up to month 3)	nd	+ (≥ year 2)
Cerebrospinal Fluid	nd	nd	nd	nd
Urine	nd	nd	nd	nd
PBMC	N/A	+ (≥ month 1)	pending	nd
Lymph nodes	N/A	+ (≥ year 2)	pending	nd
Spleen	N/A	+ (≥ year 2)	pending	+ (≥ year 1)
Liver	N/A	+ (≥ year 2)	+ (≥ year 2)	nd
Gonads	N/A	+ (up to month 3)	nd	nd

Humoral response	α-transgene	pending
	α-capsid	**+/-**
Cellular response	α-transgene	pending
	α-capsid	pending

nd = not done

Mode of delivery:

Intramuscular

Animal Model:

Primate

rAAV serotype:

1, 2, 8

rAAV dose (total):

$4x10^{11}$vg to $1,4x10^{13}$vg

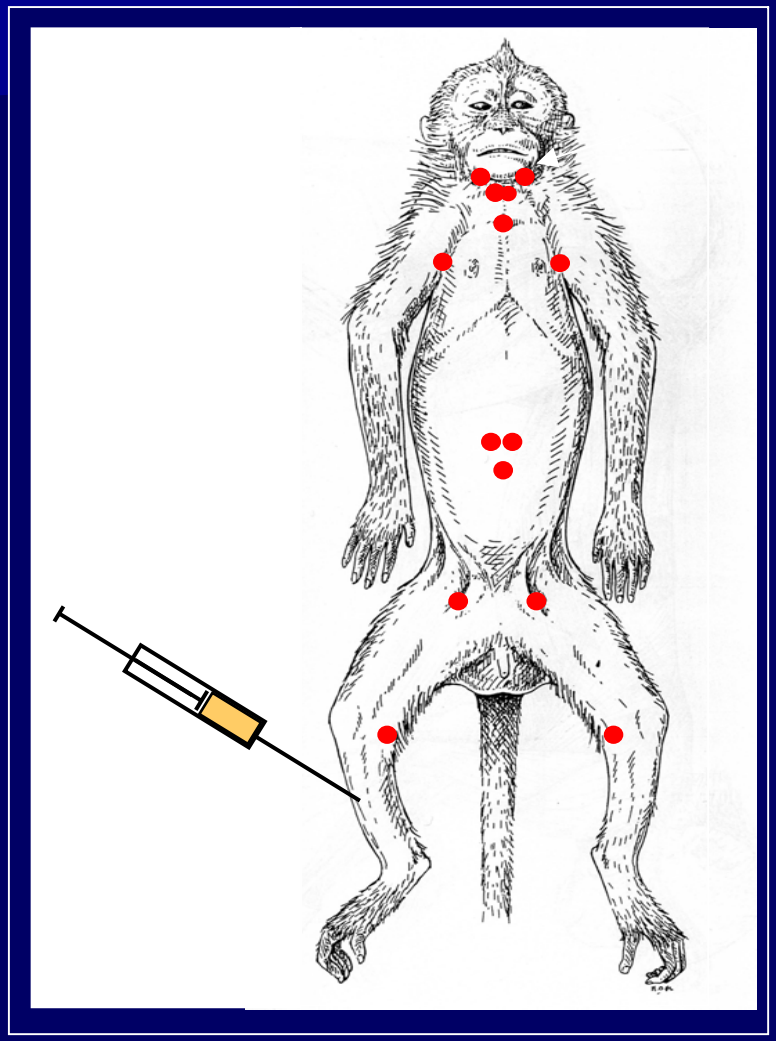
Favre *et al.*,
Mol. Ther., 2001
Chenuaud *et al.*,
Unpublished data
Toromanoff *et al.*,
Manuscript in preparation

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	5 to 50 vg/cell (≥ year 3)	+ (≥ year 3)	+ (≥ year 5)
Serum	+ (up to day 7)	+ (up to month 1,5)	nd	+ (≥ year 5)
Cerebrospinal Fluid	nd	nd	nd	nd
Urine	nd	+ (up to day 6)	nd	nd
PBMC	N/A	+ (up to month 15)	pending	nd
Lymph nodes	N/A	+ (≥ year 3)	+ (≥ day 20)	nd
Spleen	N/A	+ (≥ year 3)	pending	+ (≥ year 2)
Liver	N/A	+ (≥ year 3)	+ (≥ year 3)	nd
Gonads	N/A	+ (up to month 3)	nd	nd

Humoral response	α-transgene	+
	α-capsid	+
Cellular response	α-transgene	+
	α-capsid	+

nd = not done

One single IM injection of rAAV1, rAAV2, rAAV8 in primate results in widespread detection of vector genome in lymph nodes for years



- Positive lymph nodes

Mode of delivery:

Intrathymic

Animal Model:

Primate

rAAV serotype:

8

rAAV dose (total):

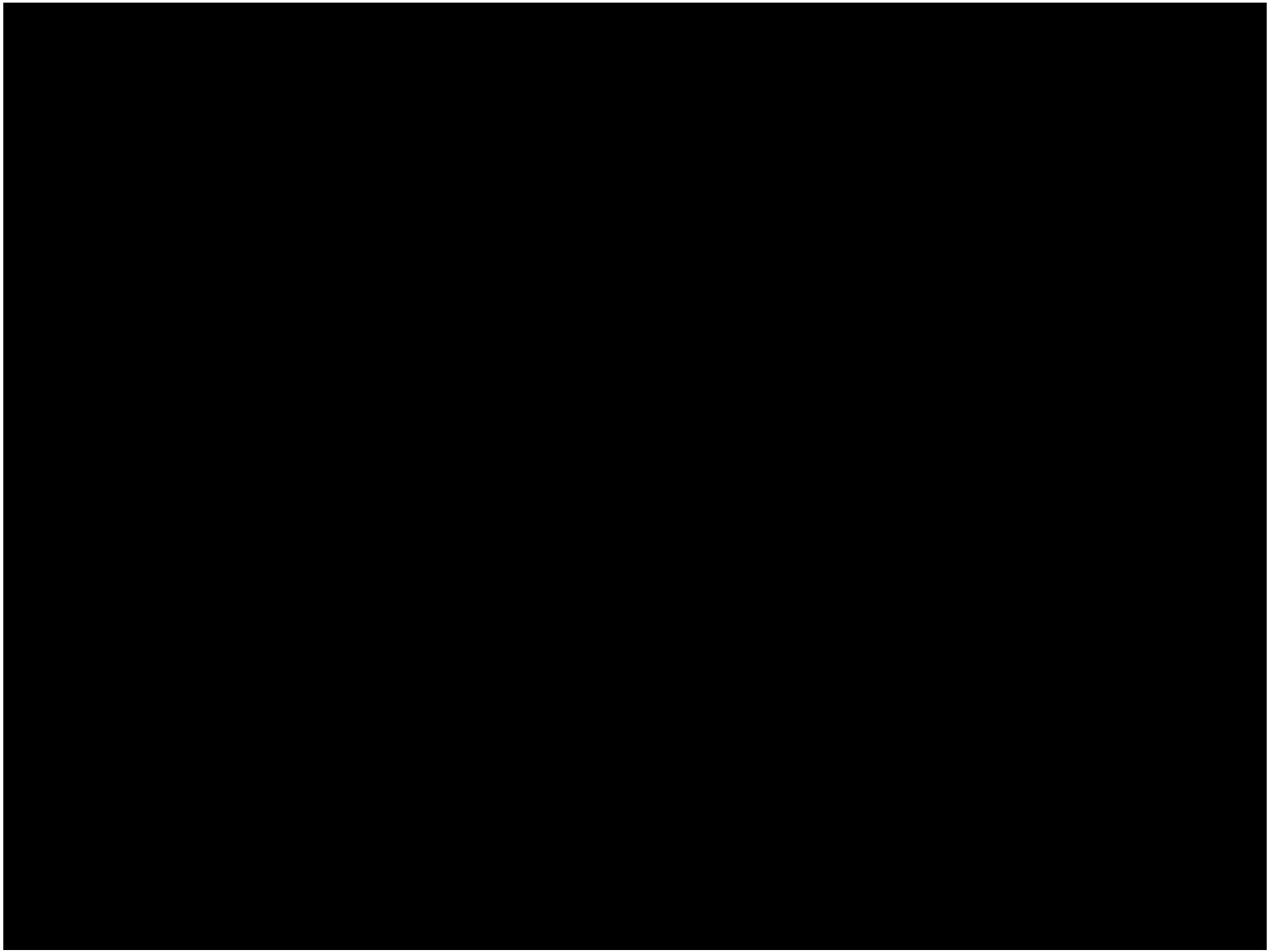
$2x10^{13}$vg

Moreau *et al.*,
Manuscript in preparation

	Infectious particles	Vector genome	Transgene mRNA	Transgene product
Target organ	N/A	+ (≥ day 10)	nd	+ (≥ day 10)
Serum	pending	pending	nd	nd
Cerebrospinal Fluid	nd	nd	nd	nd
Urine	nd	nd	nd	nd
PBMC	N/A	0,02 vg/cell (≥ month 1)	pending	-
Lymph nodes	N/A	0,1 vg/cell (≥ month 1)	pending	-
Spleen	N/A	6 vg/cell (≥ month 1)	pending	+ (≥ month 1)
Liver	N/A	61 vg/cell (≥ month 1)	pending	+ (≥ month 1)
Gonads	N/A	nd	nd	nd

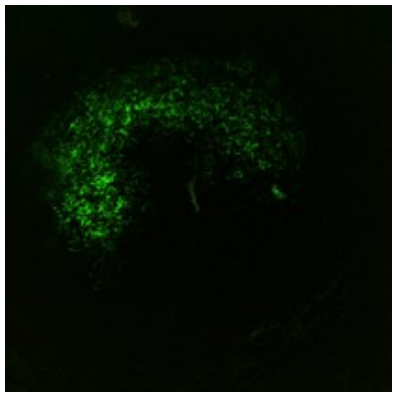
Humoral response	α-transgene	pending
	α-capsid	pending
Cellular response	α-transgene	pending
	α-capsid	pending

nd = not done

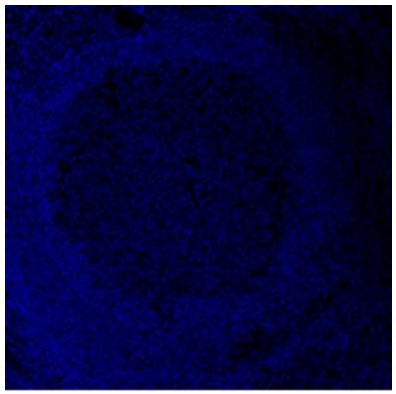


Primate Spleen, Day 30 pi: (Intrathymic injection / rAAV8-PGK-GFP)

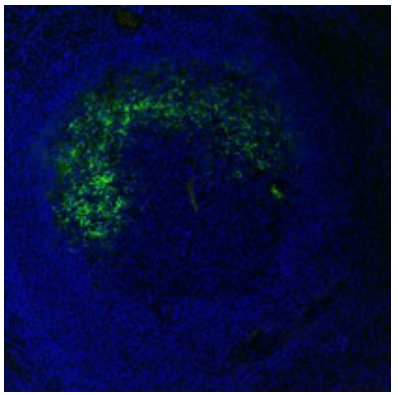
GFP



Nuclei

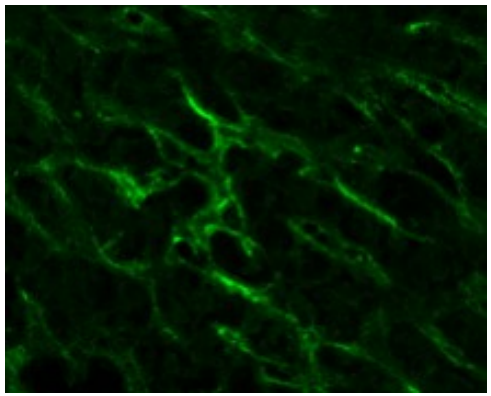


Merge

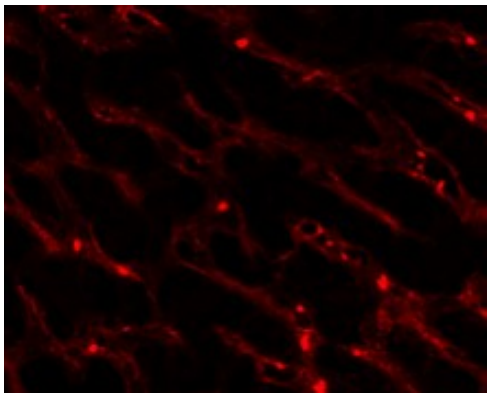


Primate Liver, Day 30 pi: (Intrathymic injection / rAAV8-PGK-GFP)

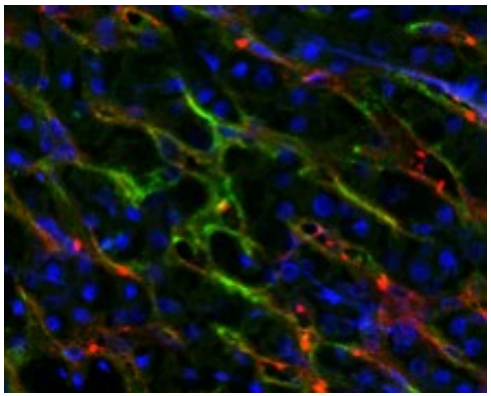
GFP



Endothelial cells



Merge + Nuclei



Moreau *et al.*, Manuscript in preparation

And in other labs?

		Jacobson *et al.*, HGT, 2006 + Mol. Ther., 2006
Mode of delivery		Subretinal
« Animal » model		Dog / Primate
rAAV serotype		2
rAAV dose		$1,5x10^{8}$ to $4,5x10^{12}$ vg
Transgene expression		**+** (≥ month 3)
vg	**Target organ**	**+** (≥ month 3)
	Serum	nd
	Cerebrospinal Fluid	nd
	Urine	nd
	PBMC	nd
	Lymph nodes	**+** (≥ month 3)
	Spleen	**-**
	Liver	**-**
	Gonads	**-**
Humoral response	α-transgene	nd
	α-capsid	**+**
Cellular response	α-transgene	nd
	α-capsid	nd

		Monahan *et al.*, Gene Ther., 1998; Chao *et al.*, Gene Ther., 1999	Kay *et al.*, Nat. Gen., 2000; Manno *et al.*, Blood, 2003; Jiang *et al.*, Mol. Ther., 2006	Brantly *et al.*, HGT, 2006
Mode of delivery		Intramuscular	Intramuscular	Intramuscular
« Animal » model		Dog	Human	Human
rAAV serotype		2	2	2
rAAV dose		$1,2x10^{12}$ to $6x10^{12}$ vg	$1,4x10^{13}$ to $7x10^{14}$ vg	$2,1x10^{12}$ to $6,9x10^{13}$ vg
Transgene expression		+ (≥ month 8)	+ (≥ year 3,7)	+ (up to month 1)
vg	**Target organ**	+ (≥ month 8)	4,1 vg/cell (≥ year 3,7)	nd
	Serum	nd	+ (up to month 3)	+ (up to day 14)
	Cerebrospinal Fluid	nd	nd	nd
	Urine	nd	+ (up to day 1)	nd
	PBMC	nd	nd	+ (up to day 14)
	Lymph nodes	+ (≥ month 8)	nd	nd
	Spleen	+ (≥ month 8)	nd	nd
	Liver	+ (≥ month 8)	nd	nd
	Gonads	-	- (semen)	- (semen)
Humoral response	α-transgene	-	nd	-
	α-capsid	+	+	+
Cellular response	α-transgene	+ ?	nd	-
	α-capsid	+ ?	nd	-

		Davidoff *et al.*, Mol. Ther., 2005; Nathwani *et al.*, Blood, 2006; Jiang *et al.*, Blood, 2006	Manno *et al.*, Nat. Med., 2006	Nathwani *et al.*, Blood, 2007
Mode of delivery		Intraportal	Intraportal	Intravenous
« Animal » model		Primate	Human	Primate
rAAV serotype		2, 5, 8	2	8
rAAV dose		$2x10^{12}$ to $1x10^{14}$ vg	$5,6x10^{12}$ to $1,4x10^{14}$ vg	$5x10^{12}$
Transgene expression		+ (≥ month 6)	+ (up to month 2)	+ (≥ month 13)
vg	**Target organ**	16 vg/cell (≥ month 6)	nd	29 to 62 vg/cell (≥ month 1)
	Serum	nd	+ (up to month 3)	+ (up to day 3)
	Cerebrospinal Fluid	nd	nd	nd
	Urine	nd	+ (up to month 1)	nd
	PBMC	nd	+ (up to month 4,5)	nd
	Lymph nodes	nd	nd	nd
	Spleen	2 vg/cell (≥ month 6)	nd	+ (≥ month 1)
	Liver	= Target Organ	= Target Organ	= Target Organ
	Gonads	0,02 vg/cell (≥ month 6)	+ (semen, up to month 4)	+ (≥ month 1)
Humoral response	α-transgene	+	-	+
	α-capsid	+	+	+
Cellular response	α-transgene	nd	-	nd
	α-capsid	nd	+	nd

Conclusions

Regardless of *the mode of delivery, the serotype and the dose,*
in situ administration of rAAV in large animal models and patients is associated with :

- extra target shedding
- presence of infectious rAAV in the circulation with slow clearance (days)
- constant detection of vector genome at distant sites including the *immune system* and the *liver*

These findings should be discussed in the context of the α-capsid and α–transgene host response as well as the potential hepatocarcinogenic effect of rAAV.

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