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Gene Therapy: Early Clinical Development Challenges

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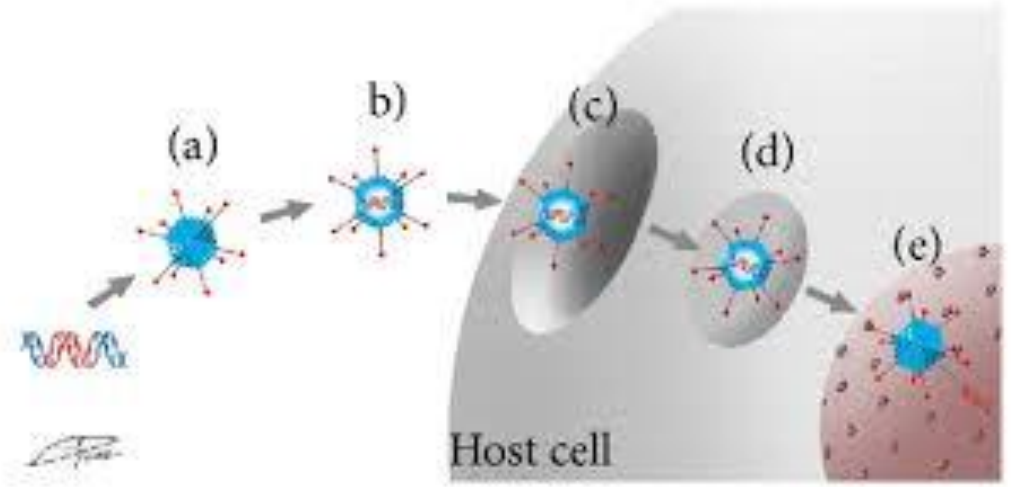
What's different about gene therapy: PK may not apply

- The therapeutic agent is encoded as DNA that is delivered by a viral capsid which must un-coat in the nucleus to release a plasmid that can transcribe a therapeutic RNA.
- The transcribed RNA can code for a protein (or peptide or antibody) and raise its levels or it can cause RNA interference and diminishes levels of the target.
- The administered agent is a vector genome but the ultimate pharmacology is downstream of the molecule that is administered.
- While there can be a dose/response relationship, a conventional PK approach of relating the kinetics of the administered molecule to a therapeutic responses or side effects is not applicable.

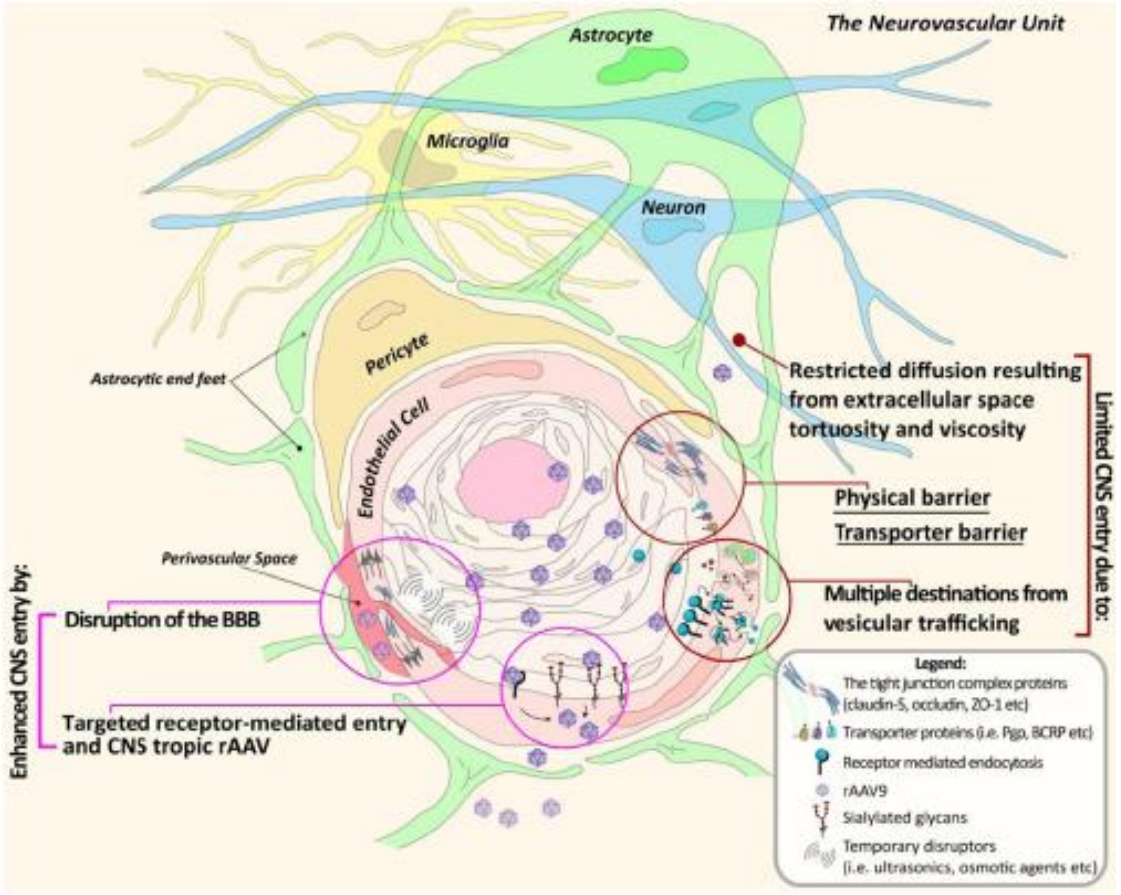


What's different about gene therapy: Route of administration

Oral administration - capsids don't survive

Intravenous administration

- **Upsides**
  - non-invasive
  - could reach the entire CNS
- **Downsides**
  - doses are very high
  - immune responses are more likely
  - BBB and tropism could hamper reaching the targeted cells
  - systemic exposure could increase the likelihood of off-target effects.



From Maguire et al, 2014

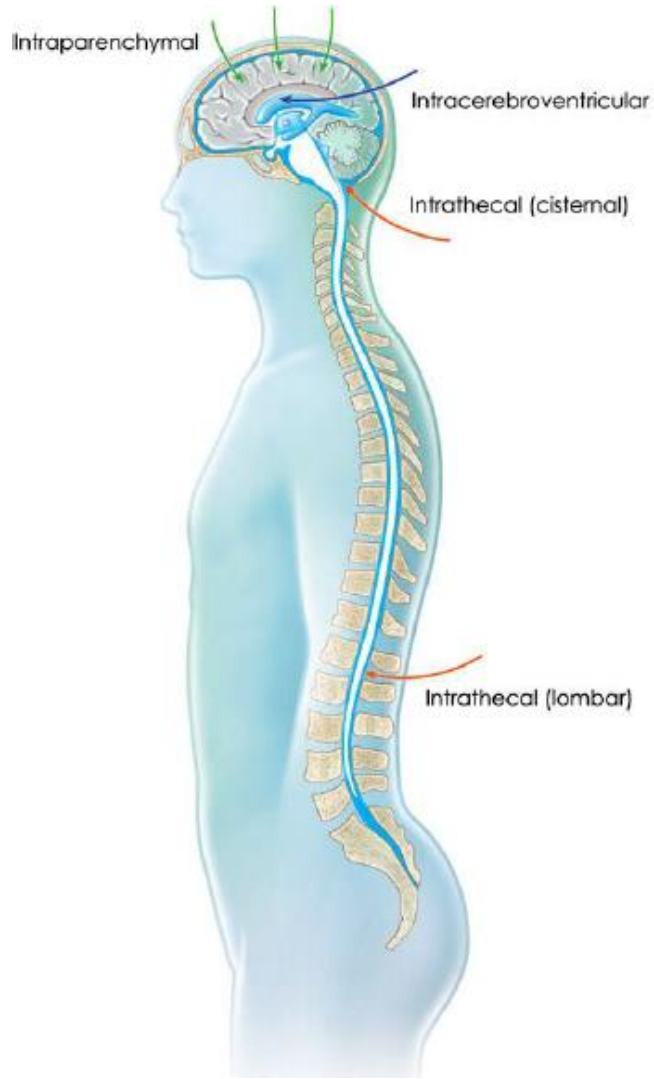
What's different about gene therapy: Route of administration

Intrathecal administration

- **Upsides**
  - Well tolerated
  - lower doses
  - reduced systemic exposure
- **Downsides**
  - exposure may be best near the site of administration and closer to the surface.

Intraparenchymal administration

- **Upsides**
  - doses can be very small
  - can precisely target specific brain regions
  - unlikely to elicit an immune response
- **Downsides**
  - Requires specialized neurosurgery, devices
  - broad CNS distribution can be difficult



From Hocquemiller et al, 2016

What's different about gene therapy: Route of administration

Table 1. *Clinical Trials*

	Injection site	Disease	Clinical trial	Inclusion	Serotype	Transgene	Promoter[a]	Dose, min vg	Dose, max vg	Volume, µL	Speed, µL/min	IS	Status	Identifier	Ref.
Intraparenchymal	WM ($n=6$)	Can	Phase I	13	2	ASP	NSE	9×10^{11}		900	2	NA	C	NA	15
	WM ($n=12$)	LINCF	Phase I	11	2	CLN2	CAG	1.8×10^{12}	3.2×10^{12}	600	2	NA	C	NCT00151216	17
	WM ($n=12$)	LINCF	Phase I/II	16	rh10	CLN2	CAG	2.85×10^{11}	9×10^{11}	1800	2	NA	O	NCT01414985	NA
	WM ($n=12$)	MPS IIIA	Phase I/II	4	rh10	SGSH	PGK	7.2×10^{11}		720	0.5	Y	C	NCT01474343	16
	WM ($n=12$)/ Cer ($n=4$)	MPS IIIB	Phase I/II	4	5	NAGLU	PGK	4×10^{12}		960	0.5	Y	O	ISRCTN19853672	NA
	WM ($n=12$)	MLD	Phase I/II	5	rh10	ARSA	CAG	1×10^{12}	4×10^{12}	NA	NA	NA	O	NCT01801709	NA
	StN ($n=2$)	Par	Phase II	16	2	GAD	CAG	2×10^{12}		70	0.23	NA	C	NCT00643890	162
	Str ($n=4$)	Par	Phase I	10	2	AADC	CMV	9×10^{10}	3×10^{11}	200	1	N	C	NCT00229736	163
	Put ($n=8$)	Par	Phase I&II	70	2	NTN (CERE-120)	CAG	1.3×10^{11}	5.4×10^{11}	80	2	NA	C	NCT00252850 NCT00400634	106,164
	Put ($n=6$)/ SN ($n=4$)	Par	Phase I/II	57	2	NTN (CERE-120)	CAG	9.4×10^{11}	2.4×10^{12}	360	2/3	NA	O	NCT00985517	165
	Str ($n=2$)	Par	Phase I	24	2	GDNF	CMV	9×10^{10}	3×10^{12}	NA	NA	NA	O	NCT01621581	NA
	Str ($n=2$)	Par	Phase I	10	2	AADC	NA	7.5×10^{11}	1.5×10^{12}	NA	NA	NA	O	NCT01973543	NA
	Put ($n=4$)	Par	Phase I/II	6	NA	AADC	NA	3×10^{11}	9×10^{11}	200/600	3	NA	O	NCT02418598	NA
	Put ($n=2$)	Par	Phase I	10	2	AADC	NA	NA		NA	NA	NA	O	NCT01395641	NA
	NBM ($n=4/6$)	Alz	Phase I	10	2	NGF (CERE-110)	CAG	1.2×10^{10}	1.2×10^{11}	40/80	2	NA	C	NCT00087789	79
	NA	Alz	Phase II	25	2	NGF (CERE-110)	CAG	2×10^{11}		NA	NA	NA	NA	NCT00876863	NA
IT	NA	GAN	Phase I	20	9	Gigaxonin	JeT	NA		NA	NA	NA	O	NCT02362438	NA
	Lom	CLN6	Phase I/II	6	9	CLN6	CAG	1.5×10^{13} vg/kg		NA	NA	NA	O	NCT02725580	NA
IV	PeV	SMA I	Phase I/II	15	9	SMN	CAG	6.7×10^{13}	3.3×10^{14} vg/kg	NA	NA	NA	O	NCT02122952	NA
	PeV	MPS IIIA	Phase I/II	9	9	SGSH	U1a	5×10^{12}	1×10^{13} vg/kg	NA	NA	Y	O	NCT02716246	NA

From Hocquemiller et al, 2016

What's different about gene therapy: Dosing

- **Gene therapy is delivered once**
  - Effects are durable (especially in non-dividing cells)
  - Acquired immunity makes redosing problematic, so adjustments aren't feasible currently
  - May not be able to improve pharmacology or turn off side effects in an individual other than adjusting other treatments.
  - Ethics require starting with a minimally effective dose and in the target population.
  - Early phase studies can be SAD  but not MAD 

What's different about gene therapy: Time-courses

- Onset of pharmacology is delayed as it may take weeks for the virus to un-coat, for the payload to express and reach a plateau, for secondary effects on the target to also plateau.
- Side effects could be an immediate response to the treatment, could emerge in concert with pharmacology, could emerge late if there is an immune response.
- Assessing safety and pharmacology in early phase studies must account for these timings (spacing of enrollment, timing of assessments, duration of follow-up)
- Because the treatment effects are durable, follow-up is measured in years (FDA guidance is 2-5 years for non-integrating virus, 15 for an integrating virus), beginning with the first patient treated.

What's different about gene therapy: Safety

- On target effects
- Off-target effects
  - Off-location
  - Off-mechanism
- Immune-responses
- Viral shedding

What's different about gene therapy: Immunology

Pre-existing immunity

- Pre-existing humoral or cellular immunity against a capsid could cause an immediate immune response or block treatment effects.
- Anti-capsid neutralizing antibodies (NAbs) are a subset of anti-capsid antibodies that prevent therapeutic transfection.
- Assays essential to screen animals for use in non-clinical studies to insure validity.
- Screening potential trial participants to exclude those with immunity, depending on ROA.
  - Low serum (1:5) titers have been associated with reduced efficacy for systemic gene therapies.
  - IgG in CSF is 12-1200X lower in children, 300X lower in adults so even high serum titers may be OK for IT or IP delivery

What's different about gene therapy: Immunology

NAb Seroprevalence

- AAV1 NAbs in 15-50%
- AAV2 NAbs in 30-60%
- AAV7, AAV8, AAV9 NAbs in 15-30%
- AAVrh10 in up to 60%
- Nab cross reactivity between capsids is frequent because of high sequence homology.

Anti-AAV Seroprevalence

- AAV1 Abs in 70%
- AAV2 Abs in 70%
- AAV6 Abs in 45%
- AAV9 Abs in 45%
- AAV8 Abs in 38%.

TABLE 1. PREVALENCE OF NEUTRALIZING ANTIBODIES AGAINST AAV SEROTYPES

Study	*Dilution*	*AAV1*	*AAV2*	*AAV5*	*AAV6*	*AAV7*	*AAV8*	*AAV9*
Boutin *et al.*, 2010	1/20	50	59	3	37		19	33
Chirmule *et al.*, 1999	1/20 (?)		32					
Murphy *et al.*, 2009	1/3.1		38					
Calcedo *et al.*, 2009; Australia	1/20	30	35			29	27	
Calcedo *et al.*, 2009; Europe	1/20	27	35			25	22	
Calcedo *et al.*, 2009; Africa	1/20	43	56			31	31	
Calcedo *et al.*, 2009; United States*	1/20	20	28			12	14	
Halbert *et al.*, 2006*			30	18	30	14	30	
Parks *et al.*, 1970	1/10		40					
Blacklow *et al.*, 1968	1/10		40					
Ito *et al.*, 2009	1/20		40					
Moss *et al.*, 2004	?		32					
Wagner *et al.*, 2002	1/20		22					
Erles *et al.*, 1999*			50	50				
Veron *et al.*, 2012	1/2	59						
Mingozzi *et al.*, 2012a	1/10		82	27	64		50	
	1/3.1		100	36	91		90	

The numbers in the columns of specific AAV serotypes indicate the percentage of subjects whose serum inhibited transduction by ≥50% at the indicated serum dilution.
*Approximate values.

Jeune et al 2013

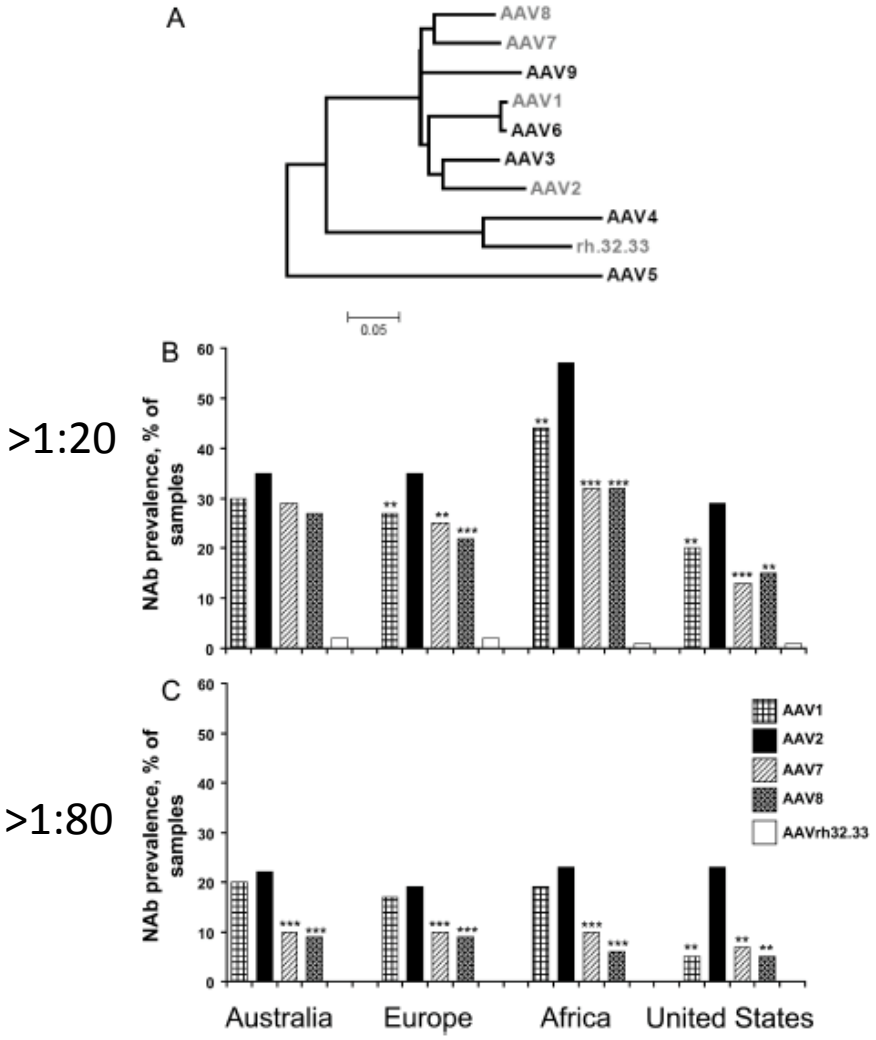
TABLE 1. Average prevalence of NAb (titer of ≥1:20) by age in anonymous serum samples from Children's National Medical Center

Group	Age (yr)	No. of samples: Tested	No. of samples: Positive	% prevalence	Relative prevalence	95% confidence interval	*P* value
Infants[a]	<1	175	31	15			
Toddlers	1–<3	83	13	13.5	0.9	0.49, 1.64	0.72
Children Adolescents	3–18	350	96	21.5	1.43	0.99, 2.07	0.052

[a] Reference group for comparisons of relative prevalence.

Calcedo et al 2011

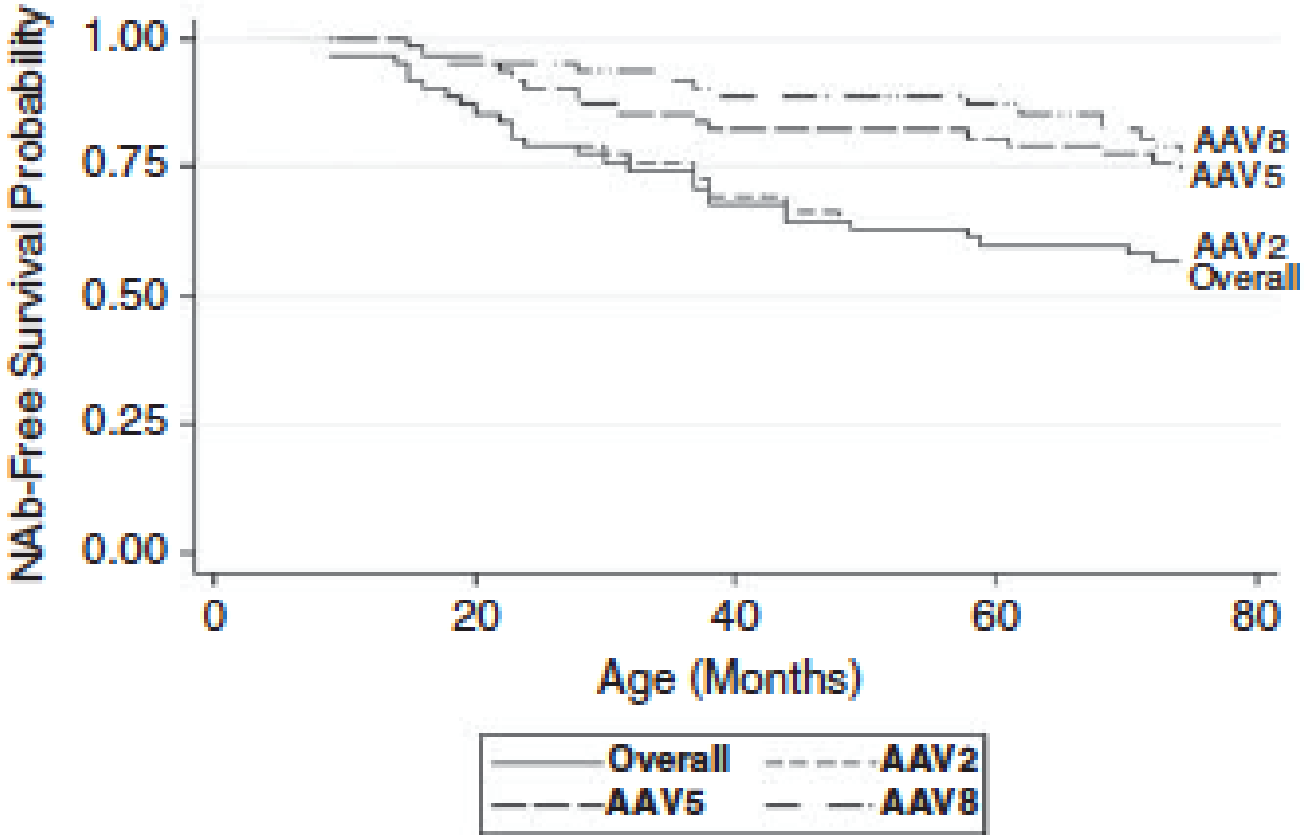
What's different about gene therapy: Immunology



>1:20

>1:80

Calcedo et al 2009



Narkbunnam et al 2011

What's different about gene therapy: Immunology

Mitigation strategies for pre-existing immunity

- Selection of naïve subjects
- Select or engineer viral subtypes with lower sero-prevalence of NAbs
- Plasmapheresis (for titers < 1:100) or immuno-absorption
- Transient immunosuppression (rituximab, cyclosporine A, methotrexate, mycophenolate, bortezomib)
- Isolated perfusion and saline flushing (not for CNS)
- Competition with empty capsids

What's different about gene therapy: Immunology

Acquired immunity

- Capsid exposure will lead to the development of immunity
- Transgene product immunity could develop depending on the 'foreignness'
- Immune attack on tissues that can present antigen can cause damage and loss of the gene therapy if its presence is cleared from the targeted tissue.
  - Monitor with assays for humoral and cellular immunity
  - Immune response in toxicology studies may not be predictive of responses in humans
  - Consider immune-suppression depending on the route of administration.
  - Monitor pharmacodynamics to assess durability of expression

What's different about gene therapy: Biomarkers

Biomarkers – fit for purpose

- **Diagnostic**: Neutralizing antibodies
- **Shedding**: Capsid
- **Target engagement**: RNAs (shRNA, miRNA, mRNA…)
- **Response**: Targeted protein
- **Safety** (?): Activated T-cells (Elispot), cytokines…

What's different about gene therapy: Study design

Since gene therapies are durable, typical Phase 1-3 study progression from safety/PK/PD to preliminary efficacy to definitive efficacy does not apply well.

- Every treated patient contributes to the long-term accumulation of safety and efficacy data.
- For neurodegenerative or other progressive diseases, the earliest patients treated can be the most informative about efficacy since follow-up is longest.
- Early inclusion of controls and blinding can maximize the contribution of all the treated patients.
- Adaptive designs may be especially applicable to enable efficient accumulation of safety and efficacy data.
- Early regulatory discussions about how to demonstrate efficacy and access accelerated approval mechanisms

What's different about gene therapy: Ethics

- Cannot treat healthy controls during early development.
- The dose should always have the potential to provide benefit.
- Participation in a gene therapy trial could affect participation in other clinical trials.
- Consent process should inform about these issues and also temper expectations at a time when there are such high hopes for gene therapy.

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What's different about gene therapy: Questions?