MedImmune

Two Approaches to Potency Bioassay Analysis

Lanju Zhang, Ph.D.
Harry Yang, Ph.D.
MedImmune LLC

May 26, 2010

Outline

- Potency bioassays
- Two approaches to analysis
  - Linear case
  - 4PL
- Conclusions

Introduction: Potency in Bioassays

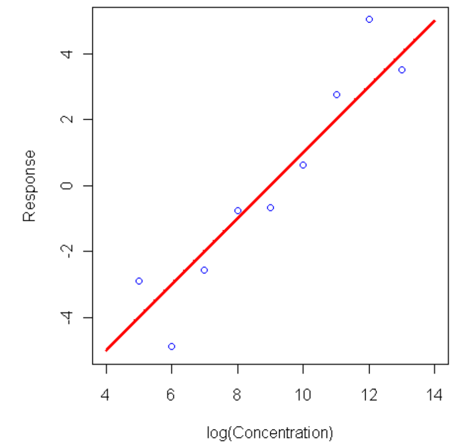
- Bioassay (USP Chapter <1046>)
  - Measurement of the effectiveness of a compound by its effect on animals or cells in comparison with a standard preparation
- Potency (USP Chapter <1046>)
  - A quantitative measure of biological activity based on the attribute of the product linked to the relevant biological properties.
- Mathematically, $F(x) = F(\rho x)$, where x is the concentration and ρ is the *relative potency*;

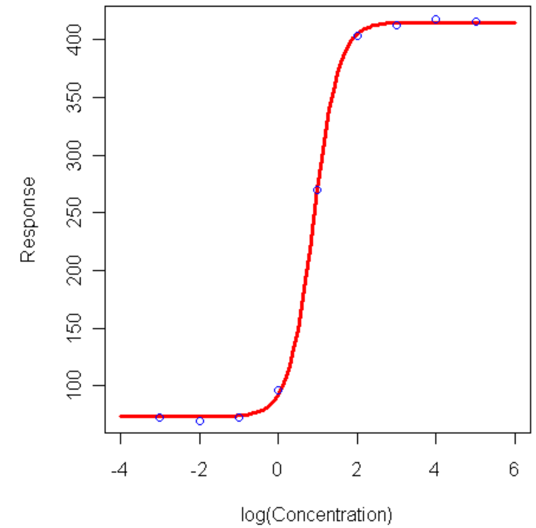
Introduction: the Models

- Models for the function *F*
  - Linear: $y = \alpha + \beta z$
    - *Z* is the log(concentration)
  - Non-linear (4-PL):

$$y = D + \frac{D - A}{1 + \exp[B(z - \log(C))]}$$

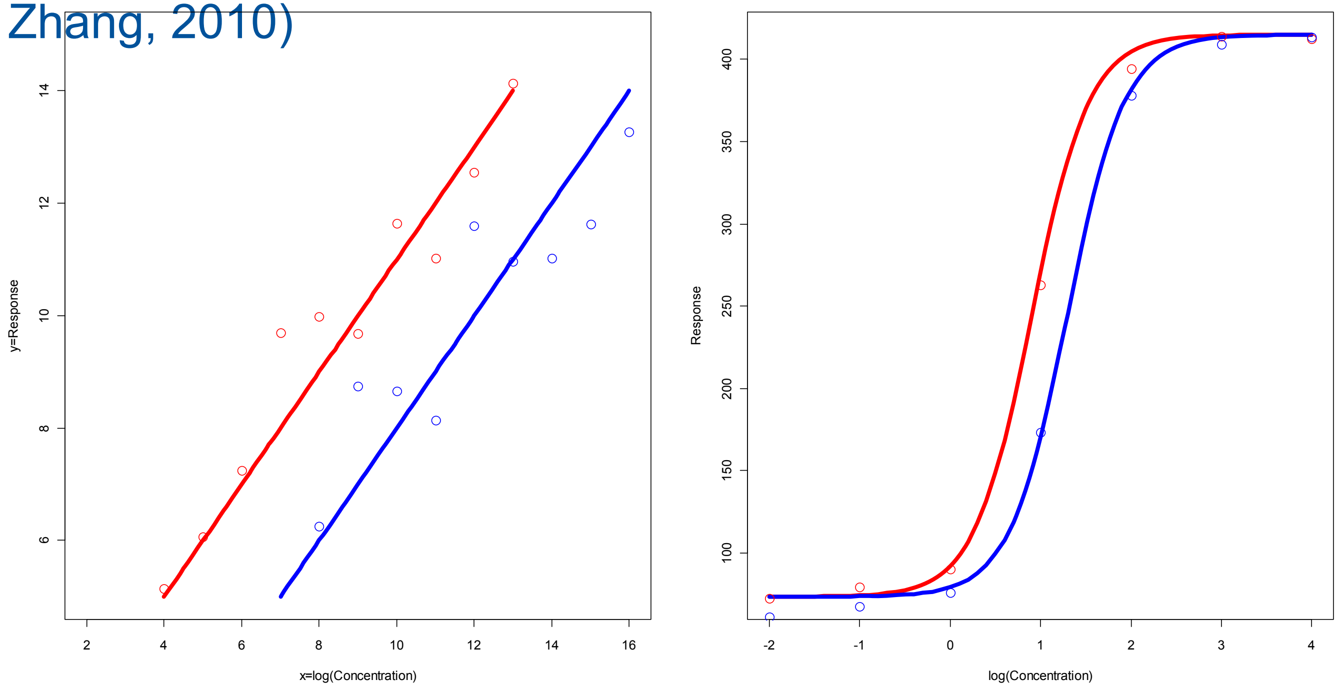
    - D: upper asymptotes
    - A: lower asymptotes
    - C: EC_{50}
    - B: slope parameter
    - 5-PL could be used to account for asymmetry





Introduction: Parallelism

- Parallelism/similarity requirement
  - Mathematically, $F(x) = F(\rho x)$
- Parallelism test:
  - Significance (Gottschalk and Dunn, 2005)
  - Equivalence (Hauck et al, 2005, Jonkman and Sidik, 2009, Yang and Zhang, 2010)



Defining Relative Potency: Linear Case

- Separate models

$$y = \alpha_T + \beta z + e$$

$$y = \alpha_R + \beta z + e$$

- Relative potency

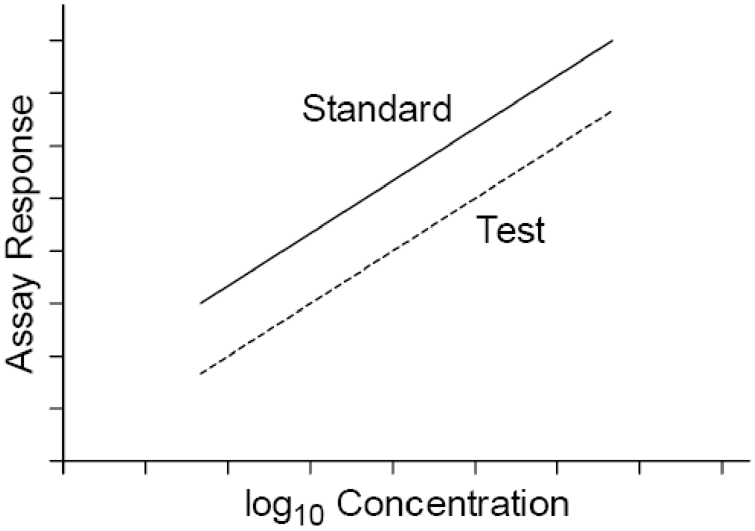
$$\rho = \exp\left(\frac{\alpha_T - \alpha_R}{\beta}\right)$$

- Combined model

$$y = \alpha + \beta_1 T + \beta_2 z + e$$

- Estimation of relative potency

$$\rho = \exp\left(\frac{\beta_1}{\beta_2}\right)$$



**Courtesy: USP Chapter <111> revision (2007 fall version)

Defining Relative Potency: 4-PL Case

- The models

$$y = D + \frac{D - A}{1 + \exp[B(z - \log(C_T))]}, \quad y = D + \frac{D - A}{1 + \exp[B(z - \log(C_R))]}$$

- Relative potency

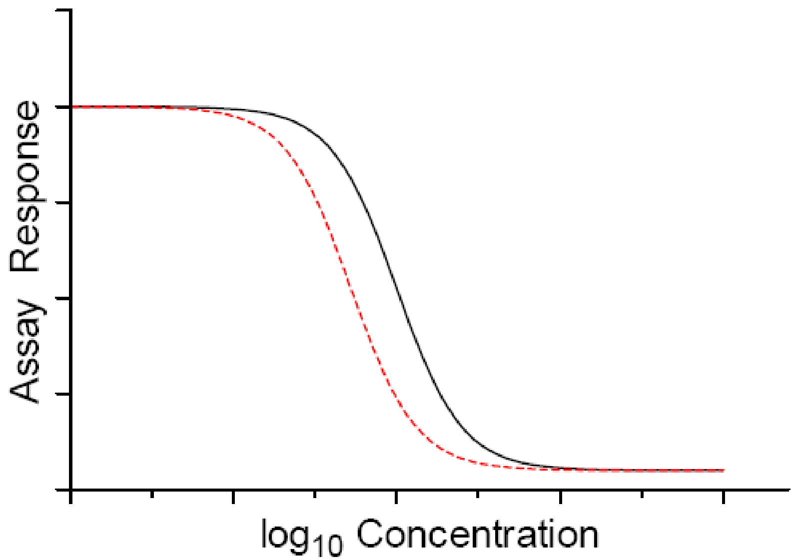
$$\rho = \frac{C_T}{C_R}$$

- Combined model

$$y = D + \frac{D - A}{1 + \exp[B(z - \log(C) + rT)]},$$

- Estimation of relative potency

$$\rho = \exp(r)$$



**Courtesy: USP Chapter <111> revision (2007 fall version)

Estimation Method 1: 4-PL Case

Data: Reference

Data: Sample

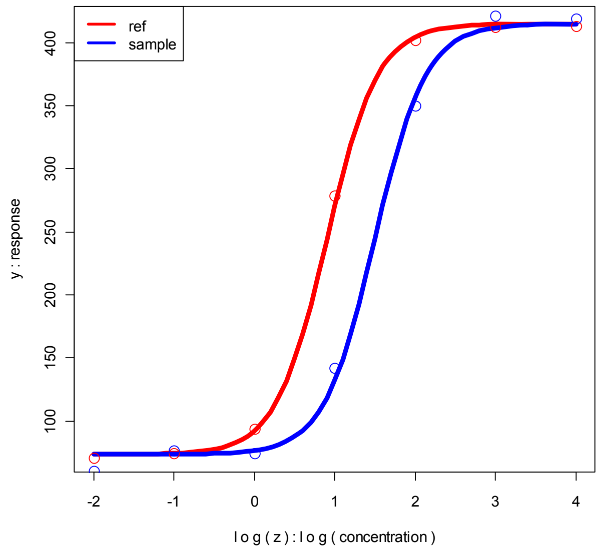
fitted by a single 5-parameter model:

$$y = D + \frac{A - D}{1 + \left(\dfrac{z}{C_1 \cdot \left(\dfrac{C_2}{C_1}\right)^T}\right)^B} + \varepsilon,$$

T=0 for reference and *T=1* for sample, i.e., common *A*, *B* and *D*, and different *C.*

Thus, $\hat{\rho} = \hat{C}_1 / \hat{C}_2$ (Least Square Estimation).

•Method 1:
pooled analysis (USP recommended)



Estimation Method 2: 4-PL Case

Data: Reference	$\hat{A}_1$, $\hat{B}_1$ and $\hat{D}_1$ carried over from reference to sample →	Data: Sample	Method 2: unpooled analysis

fitted by a 4 - PL model :

$$y = D_1 + \frac{A_1 - D_1}{1 + (\frac{x}{C_1})^{B_1}} + \varepsilon,$$

and get estimates $\hat{A}_1$, $\hat{B}_1$, $\hat{C}_1$ and $\hat{D}_1$.

fitted by a constrained 4 - PL model :

$$\boldsymbol{y} = \hat{\boldsymbol{D}}_1 + \frac{\hat{\boldsymbol{A}}_1 - \hat{\boldsymbol{D}}_1}{1 + (\frac{\boldsymbol{x}}{\boldsymbol{C}_2})^{\hat{\boldsymbol{B}}_1}} + \boldsymbol{\varepsilon},$$

with $\hat{\boldsymbol{A}}_1, \hat{\boldsymbol{B}}_1, \hat{\boldsymbol{D}}_1$ fixed and get estimate $\hat{C}_2$.

$$\hat{\rho} = \hat{C}_1 / \hat{C}_2$$

MedImmune

Two Estimation Method: Linear Case

Data: Reference | Data: Sample → One model

Method 1: pooled analysis

Data: Reference $\xrightarrow{\beta \text{ carried over from reference to sample}}$ Data: Sample

Method 2: unpooled analysis

MedImmune

Question

- In practice
  - For 4-PL case, a curve is fit for reference standard; another curve is fit for sample using parameters obtained in the first fit except *C*
    - In SoftMax, often by choosing constrained option
    - In Prism, often by choosing hook option
  - Linear case
    - Easy to fit separate models in Excel
- Statistically
  - If variance is the same for sample and reference standard, a combined model should be fit for maximum efficiency
- **QUESTION:**
  - ***How much do we lose if we fit separate models compared to combined model?***

Linear Case: parameter estimates

Method 1: separate models

$$\hat{\mu}_{\mathrm{r}} = \overline{\mathrm{y}}_{\mathrm{r}} - \hat{\beta}\,\overline{\boldsymbol{x}}$$

$$\hat{\mu}_{s} = \overline{\mathrm{y}}_{s} - \hat{\beta}\,\overline{\boldsymbol{x}}$$

$$\hat{\rho} = \exp\left(\frac{\hat{\mu}_s - \hat{\mu}_r}{\hat{\beta}}\right) = \exp\left(\frac{\overline{\mathrm{y}}_s - \overline{\mathrm{y}}_{\mathrm{r}}}{\hat{\beta}}\right)$$

$$\text{variance}(\overline{\mathrm{y}}_s - \overline{\mathrm{y}}_{\mathrm{r}}) = 2\sigma^2 / \boldsymbol{n}$$

$$\text{variance}(\hat{\beta}) = \sigma^2 / \boldsymbol{S}_X$$

Method 2: Combined model

$$\boldsymbol{y} = \mu + \alpha\,\boldsymbol{t} + \beta\,\boldsymbol{x} + \varepsilon$$

$$\hat{\alpha} = \hat{\mu}_s - \hat{\mu}_r$$

$$\hat{\rho} = \exp\left(\frac{\hat{\alpha}}{\hat{\beta}}\right)$$

$$\text{variance}(\hat{\alpha}) = 2\sigma^2 / \boldsymbol{n}$$

$$\text{variance}(\hat{\beta}) = \sigma^2 / (2\boldsymbol{S}_X)$$

$$\hat{\alpha} \perp \hat{\beta}$$

Relative potency?

- Log (relative potency) is a ratio of two normal variables
- Example (Hauck et al, 2005)
  - x=log(c(10,20,40,80,160))
  - y=c(0.11,0.23,0.48,0.96,1.92)

$$\hat{\beta} = 0.625, \ \ \boldsymbol{Sx} = 4.8, \frac{1}{\sqrt{\boldsymbol{Sx}}} = 0.456, \frac{1}{\sqrt{2\boldsymbol{Sx}}} = 0.323$$

Table Estimation of $\log(\rho)$

$\hat{\beta}$	Method 1			Method 2		
	CV	Mean	sd	CV	mean	sd
0.625	0.73	0.78	84.3	0.52	-0.39	101
$4/\sqrt{2\boldsymbol{Sx}}$	0.35	0.58	5.58	0.25	0.43	0.64
$10/\sqrt{2\boldsymbol{Sx}}$	0.14	0.16	0.2	0.10	0.15	0.2

Relative potency?

- A new simulation (Not exact parallelism)
  - Reference slope=1
  - |Sample slope-1|< Δ

Δ	Method 1			Method 2		
	CV	Mean	sd	CV	mean	sd
0.2	0.71	3.14	59.70	0.50	1.84	16.20
	0.35	1.75	1.61	0.25	1.57	1.11
	0.14	1.53	0.47	0.10	1.51	0.40
0.1	0.71	8.15	176	0.50	0.98	57.9
	0.35	1.75	3.35	0.25	1.64	1.26
	0.14	1.53	0.45	0.10	1.50	0.40

Summary for Linear Case

- Since log(ρ) is a ratio of two normal variables
  - estimate of $\hat{\beta}$ by both methods is instable if CV of $\hat{\beta}$ is large (>1/4)
  - estimate of $\hat{\beta}$ in Method 2 is more stable than method 1 if CV of is moderate
  - Both methods performs equally well if CV of $\hat{\beta}$ if small (<1/6)

- Therefore, it is more important to have an assay with a wide response window than which analysis method to use.

- It is possible to calculate the exact variance and mean of ratio of two normal variables (Pham-Gia et al, 2006). This work is still ongoing.

4-PL Case

- Analytical comparison for 4-PL case is difficult
- Simulation studies are used
- Attempt to mimic real situations
- The evaluation criterion is MSE, accounting for both bias and variance

Mean Square Errors by Simulations

- Mean Square error:

$$\mathrm{MSE}(\hat{\theta}) = E[(\hat{\theta}-\theta)^2] = \mathrm{var}(\hat{\theta}) + \mathrm{bias}(\hat{\theta})^2$$

$$\boldsymbol{\theta} = \boldsymbol{A}, \boldsymbol{B}, \boldsymbol{C}_1, \boldsymbol{C}_{2,} \boldsymbol{D}, \boldsymbol{\rho}, \text{ and } \hat{\boldsymbol{\theta}} \text{ is}$$

$$\text{the Least Square Estimates of parameters.}$$

- For 4-PL (nonlinear), it is difficult to get the analytical expressions for the MSE's.

$$(\hat{\theta}_i - \theta)^2, i = 1,2,\ldots, N,$$

- Alternatively, use simulations to approximate them.
  - For parameter θ, simulate *N* samples and get estimates $\hat{\theta}_1, \hat{\theta}_2, \ldots, \hat{\theta}_N$. Then, the sample mean of $(\hat{\theta}_i - \theta)^2, i = 1,2,\ldots, N,$ approximates the true MSE when *N* is large (*N*=1000).

Simulations

- $$y_i = D + \frac{A-D}{1+(\frac{z_i}{C_k})^B} + e_i, \quad \sigma^2 = \mathrm{var}(e_i).$$

- Generate 1000 sets of 1 reference and 4 samples corresponding to different relative potency values. Parameters across the sets are the same.

$$A = 73454, \ B = 1.36, \ C_{ref} = 7.98, \ D = 415000$$

$\rho = C_{ref} / C_s$	Sample 1	Sample 2	Sample 3	Sample 4
	.50	.75	1.25	1.50

- 10 concentration levels $(150, 150/2^1, 150/2^2, \ldots, 150/2^9)$ and 3 replications at each level.

MedImmune

Three scenarios

- The pooled analysis recommended by USP draft has an underlying assumption of equal variances for the reference and sample. But in practice, they are rarely the same.

$$\sigma_{ref} = \sigma_{sample};$$

$$\sigma_{ref} = \sigma_{sample} \cdot 2;$$

$$\sigma_{ref} = \sigma_{sample} / 2.$$

Mean square errors of parameters

$$\text{I. } \sigma_{ref} = \sigma_{sample} = 6000 \ \ \text{(Equal variances)}$$

Pooled: USP recommended; Unpooled: currently adopted.

	Sample 1		Sample 2		Sample 3		Sample 4	
	pooled	unpooled	pooled	unpooled	pooled	unpooled	pooled	unpooled
A	1813	2968	1852	2816	2159	2896	2220	2293
B	.0356	.0527	.0363	.0518	.0368	.0512	.0369	.0513
C_1	.1994	.2334	.1886	.2268	.1932	.2358	.1891	.2262
C_2	.3864	.4499	.2514	.3010	.1529	.1868	.1197	.1472
D	2691	3302	2487	3285	2192	3353	2183	3268
$\rho=C_1/C_2$	.0121	.0121	.0179	.0179	.0303	.0303	.0368	.0368

pooled better

equal

- For A, B, C_1,C_2 and D, pooled method is better than unpooled method;
- But for ρ, the two methods have the same values of MSE.

Mean Square Errors of Parameters

$$\text{II. } \sigma_{ref} = 12000; \quad \sigma_{sample} = 6000 \ \text{(Reference more noisy)}$$

Pooled: USP recommended; Unpooled: currently adopted.

	Sample 1		Sample 2		Sample 3		Sample 4	
	pooled	unpooled	pooled	unpooled	pooled	unpooled	pooled	unpooled
A	2640	5787	3159	6144	3449	5901	3781	6101
B	.0590	.1050	.0597	.1068	.0606	.1086	.0600	.1082
C_1	.3774	.4649	.3570	.4607	.3436	.4581	.3398	.4557
C_2	.5338	.7652	.3385	.5207	.1946	.3114	.1653	.2649
D	4769	6724	4294	6995	3596	6985	3239	6817
$\rho = C_1/C_2$	.0204	.0204	.0305	.0305	.0486	.0486	.0587	.0587

pooled much better

equal

- For A, B, C_1,C_2 and D, pooled method is much better;
- But for ρ, the two methods have the same values of MSE.

Mean square errors of parameters

$$\text{III.}\ \sigma_{ref} = 6000; \quad \sigma_{sample} = 12000 \ \text{(Reference more precise)}$$

Pooled: USP recommended; Unpooled: currently adopted.

	Sample 1		Sample 2		Sample 3		Sample 4	
	pooled	unpooled	pooled	unpooled	pooled	unpooled	pooled	unpooled
A	2971	2932	3243	2905	3291	2971	3457	3010
B	.0569	.0533	.0604	.0537	.0575	.0523	.0601	.0538
C_1	.2473	.2247	.2521	.2367	.2489	.2324	.2361	.2230
C_2	.7039	.6760	.4770	.4651	.2799	.2708	.2319	.2201
D	3876	3352	3911	3469	3719	3429	3657	3361
$\rho=C_1/C_2$	.0198	.0198	.0304	.0304	.0499	.0499	.0582	.0582

unpooled better

equal

- For A, B, C_1,C_2 and D, unpooled is a little better (Caution needed);
- But for ρ, the two methods have the same values of MSE.

Real data

- Evaluate 48 Pairs of parallel reference and sample curves
- Calculate differences

	Pooled		Unpooled	
	Mean	**SD**	**Mean**	**SD**
C_ref	8.2546	1.3686	8.2523	1.3972
C_sample	9.2077	3.6133	9.2122	3.6092
ρ	0.9897	0.2890	0.9893	0.2887

Summary

- For the least square estimators of A, B, C_1,C_2 and D,
  - when the variances of the random errors of the reference are equal to /or larger than those of sample, pooled analysis has a smaller MSE than unpooled analysis;
  - otherwise, the conclusion depends on the difference of the two variances;
- But for ρ,
  - under all 3 scenarios, the two methods lead to the same values of MSE.
  - the same for a real data set

MedImmune

Conclusions

- Two methods could be used to analyze potency assays
  - Separate modeling is more convenient
  - Pooled modeling is more complicated

- Linear case
  - Pooled analysis has a better performance in terms of variance of slope estimates by theoretical reasoning and simulation
  - For log-relative potency,
    - if CV of slope estimate is large, then both methods give unstable estimate
    - if CV of slope estimate is moderate, then pooled analysis could be significantly better than separate analysis
    - If CV of slope estimate is small, then both methods give similar results
  - It is very important to have a wide response window relative to slope variability

MedImmune

Conclusions

- Two methods could be used to analyze potency assays
- 4-PL case:
  - Simulations shows that in practical situations, both methods performs similarly for estimating log relative potency

MedImmune

References

- Gottschalk PJ. and Dunn JR (2005). Measuring Parallelism, Linearity, and Relative Potency in Bioassay and Immunoassay Data. *Journal of Biopharmaceutical Statistics,* 15 437-463.
- Hauck WW, Capen RC, Callahan JD, Muth JED, Hsu H, Lansky D, Sajjadi NC, Seaver SS, Singer RR and Weisman D (2005). Assessing parallelism prior to determining relative potency. *Journal of pharmaceutical science and technology*, 59, 127-137.
- Jonkman J and Sidik K (2009). Equivalence Testing for Parallelism in the Four-Parameter Logistic Model. *Journal of Biopharmaceutical Statistics*, 19, 818-837.
- Pham-Gia T, Turkkan N and Marchand E (2006). Density of the Ratio of Two Normal Random Variables and Applications. *Communications in Statistics—Theory and Methods*, 35, 1569–1591.
- Yang H and Zhang L (2010). Evaluations of Parallelism Test Methods Using ROC Analysis. Submitted.
- USP chapter <1046>.
- USP chapter <111> revised version (2007 fall)

Acknowledgement

- Yanqing Hu
- Paul Juneau
- Iksung Cho
- Jun Kim
- Xu-Rong Jiang