Two-Stage Designs for proteomic and gene expression studies applying methods differing in costs

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Motivation

In genomic or proteomic studies we generally search for genes or proteins which explain the occurrence of a disease, the curse of a disease, ...

Limitations

- very large number of candidate genes (proteins)
- only a very small fraction of genes (proteins) show noticeable effects

Constraints

- Limit of resources: total costs C are fixed

Test Problem:

Consider

m_1 hypotheses for the mean of independent normally distributed observations μ_i with known variance σ^2

$$H_{0i}: \ \mu_i = 0 \quad \text{versus} \quad H_{1i}: \ \mu_i > 0 \quad i = 1, \ldots, m_1$$

assuming independence of observations across hypotheses

Motivation: Example

Consider an experiment with

- $m_1 = 1000$ ... number of hypotheses tests
- $C = 20000$ ... fixed total costs

Given

- $\pi_0 = 0.99$ ... proportion of true null hypotheses among all m_1 hypotheses
- $\Delta = \mu/\sigma = 0.75$ ... effect size

Conventional Single-Stage Design

- The costs per observation are set to $c_1 = 1$.
- Distribute total costs $C = 20000$ equally among the hypotheses

$$\Rightarrow 20000/1000 = 20 \text{ observations per hypothesis test}$$

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Error Rates

Notation

R ... number of rejected hypotheses
FP ... number of false positive decisions

Family Wise Type I Error Rate (FWE)

is defined as the probability of at least one Type I error:

$$FWER = P(FP \geq 1)$$

False Discovery Rate (FDR)

is the expected proportion of Type I errors among the rejected hypotheses (Benjamini and Hochberg, 1995):

$$FDR = E\left(\frac{FP}{\max\{R, 1\}}\right)$$

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Controlling the Family Wise Type I Error Rate

Bonferroni adjusted level

- Reject all hypotheses with a p-value $p_i \leq \gamma$
- $\gamma = \alpha / m_1$

Example: Single-Stage Design with 20 observations

$\alpha = 0.05$: $\gamma = 0.05/1000 = 0.00005$

Assuming an effect size of $\Delta = 0.75$ the Power (expected fraction of correctly identified effective genes/proteins) is 0.296

Because we have to adjust for multiplicity, the power of the single-stage design is very low as compared to a single hypothesis test with the same sample size.

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Controlling the False Discovery Rate (Storey 2002)

Storey's procedure to control the FDR at level α

- m_1 hypothesis tests
- Reject all hypotheses with a p-value $p_i \leq \gamma$
- To control the FDR at level α choose the maximal γ so that

$$\widehat{FDR}_\gamma(p_1, \ldots, p_{m_1}) = \frac{\gamma\,\widehat{\pi}_0 m_1}{\max(\sharp\{p_i \leq \gamma\}, 1)} \leq \alpha$$

where $\widehat{\pi}_0 = \sharp\{p_i > \lambda\}/\{(1-\lambda)m_1\}$ and $0 < \lambda < 1$

Example: Single-Stage Design with 20 observations

For $\Delta = 0.75$: $Power = 0.443$ (FWE: $Power = 0.296$)

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Single-stage designs

Single-stage designs have low power to detect existing effects.

Two-stage designs

Screen for promising hypotheses in the first stage which are further investigated in the second stage:

- Limit of resources: total costs C are fixed
- A fraction r of the resources C are used in the first stage for screening
- The remaining resources $(1 - r)C$ are used for second stage
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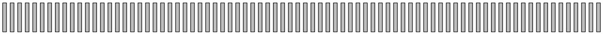
The Two-Stage Design

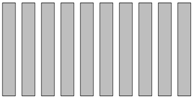
m_1 hypotheses

$$n_1 = \frac{rC}{m_1}$$



Select H_i: $p_{i1} < \gamma_1$

m_2 (random)



$$n_2 = \frac{(1-r)C}{m_2}$$

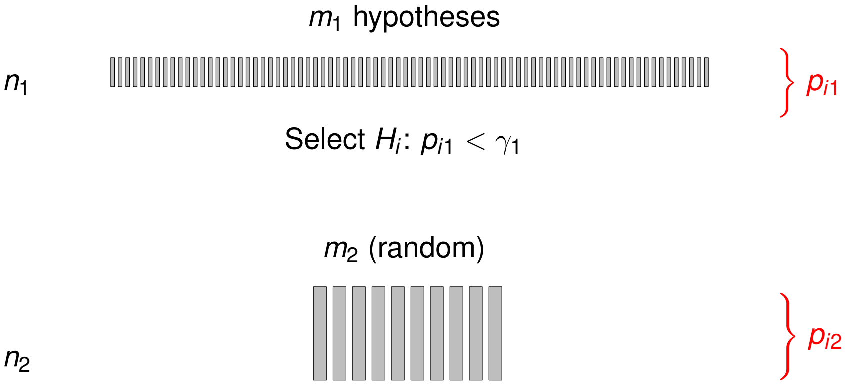
Test decision: Reject H_i: $p_{i2} < \gamma_2$

The computation of the p-value after the second stage depends on the ...

...design of the two-stage experiment

- Pilot Design
- Integrated Design

The Pilot Design



Test decision: Reject H_i: $p_{i2} < \gamma_2$

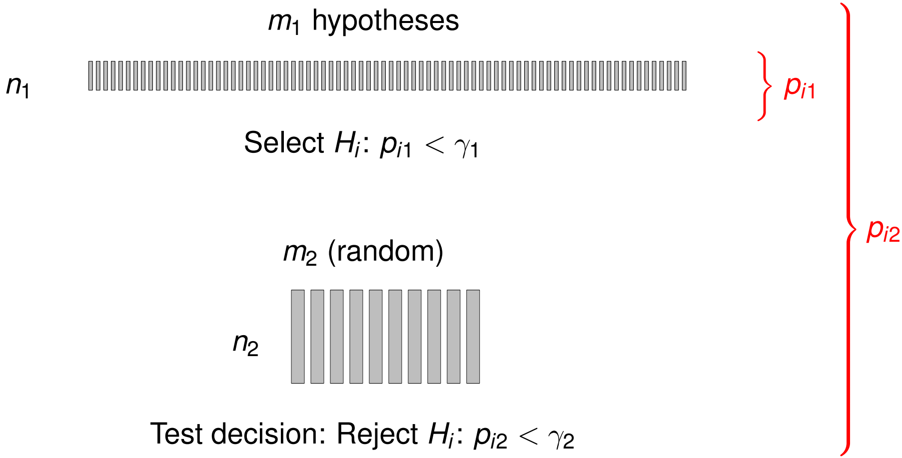
The Pilot Design

The final test decision after the second stage is only based on the second stage data:

$\rightarrow$ Sample size for the m_2 final hypothesis tests is n_2.

The correction for multiplicity is performed for the m_2 selected hypotheses using Bonferroni's correction (FWE) or Storey's procedure (FDR).

The Integrated Design



The Integrated Design

The final test decision is based on the pooled observations over both stages:

→ Sample size for m_2 final hypothesis tests is $n_1 + n_2$.
→ Price to be payed: the correction has to be performed for all initial m_1 hypotheses.

Error Rate Control

- early acceptance decision in a sequential test procedure
- define sequential p-values for all m_1 hypotheses on the Tsiatis-Rosner-Metha (1984) ordering in the sample space:
  For hypotheses early accepted we use the first stage p-value, for the final analysis we integrate over all sample path more extreme than the observed one.
- Despite than m_2 and n_2 are random the sequential p-values are independent and uniformly distributed under the null hypothesis. (see Zehetmayer et al, 2005)

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Asymptotic optimal designs

Two-stage designs

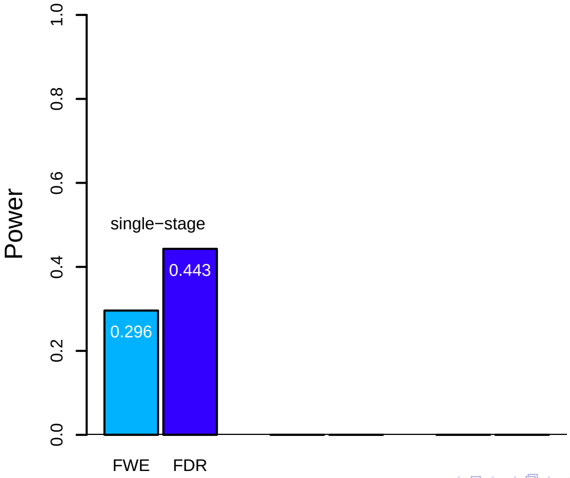
For given overall costs C, proportion of true null hypotheses π_0 and effect size $\Delta = \mu/\sigma$ the power of a two-stage design can be optimized with respect to:

- r ..fraction of total costs used in the first stage
- γ_1 ..selection boundary after first stage

Asymptotic: for $m_1 \to \infty$ and $C = d * m_1$ for $d > 0$

Asymptotic optimal designs

$C = 20000$, $m_1 = 1000$, $\Delta = 0.75$, $\alpha = 0.05$, $\pi_0 = 0.99$



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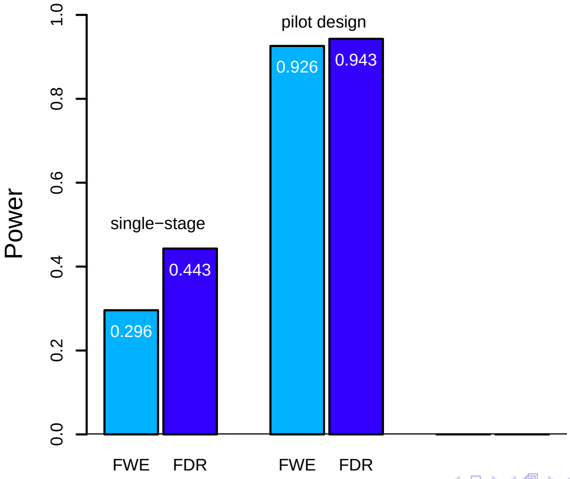

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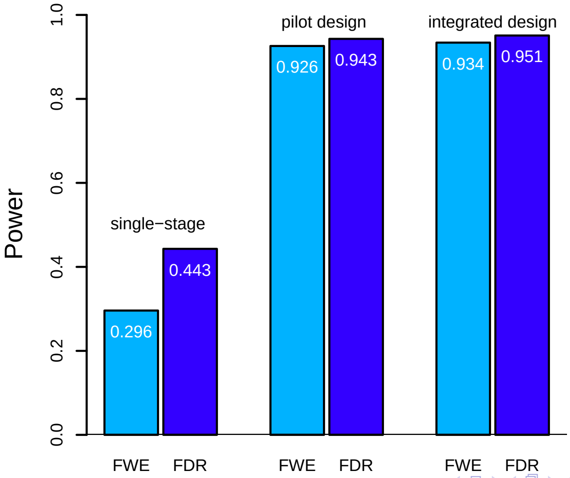

Example:

Pilot Design controlling the FWE

- 71.8 % of C are used in the first stage
- $n_1 = 20000 * 0.718/1000 \approx 14$
- $\gamma_1 = 0.092$
- $m_2 \approx$ 100 hypotheses are selected for the second stage
- $\gamma_2 \approx 0.05/100 = 0.0005$
- $n_2 = (1 - 0.718) * 20000/100 \approx 56$

In Genomic or Proteomic studies ...

Scenario 1: Different costs

Different costs per observation may arise at both stages:

- costs per observation in the first stage set to $c_1 = 1$
- cost ratio between stages $c_2 > 1$

Scenario 2: Different costs and effect sizes

There is an increasing focus on using a less accurate assay in early stages and a more accurate one in later stages:

- cost ratio $c_2 > 1$
- effect size ratio between stages $k > 1$

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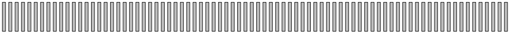
The Two-Stage Design: Scenario 1

m_1 hypotheses

effect size Δ

$$n_1 = \frac{rC}{m_1}$$

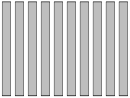


Select H_i: $p_{i1} < \gamma_1$

m_2 (random)

effect size Δ

$$n_2 = \frac{(1-r)C}{c_2 m_2}$$



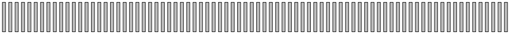
Test decision: Reject H_i: $p_{i2} < \gamma_2$

The Two-Stage Design: Scenario 2

m_1 hypotheses

effect size Δ

$n_1 = \frac{rC}{m_1}$

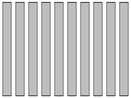


Select H_i: $p_{i1} < \gamma_1$

m_2 (random)

effect size $k\Delta$

$n_2 = \frac{(1-r)C}{c_2 m_2}$



Test decision: Reject H_i: $p_{i2} < \gamma_2$

Scenario 1: Example

Consider again the experiment with

- $m_1 = 1000$ ... number of hypotheses tests
- $C = 20000$ ... fixed total costs (limit of resources)

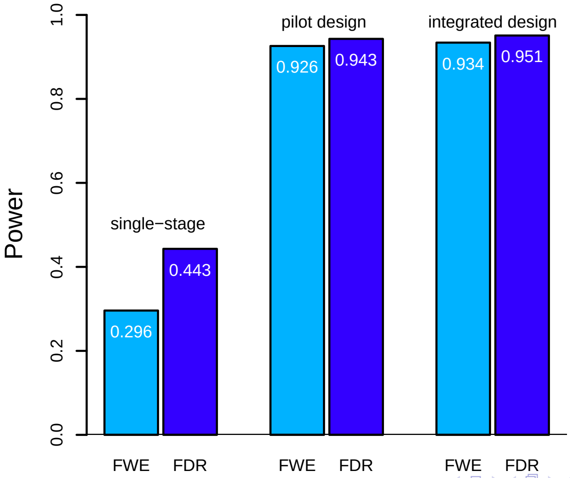
Given

- $\pi_0 = 0.99$ ... proportion of true null hypotheses among all m_1
- $\Delta = 0.75$ ... effect size

We now assume that there a cost ratio between stages $c_2 > 1$.

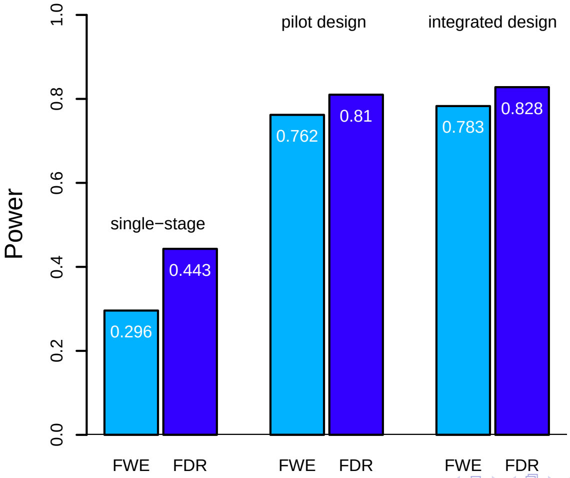
Asymptotic optimal designs

$C = 20000$, $m_1 = 1000$, $\Delta = 0.75$, $\alpha = 0.05$, $\pi_0 = 0.99$, $c_2 = 1$



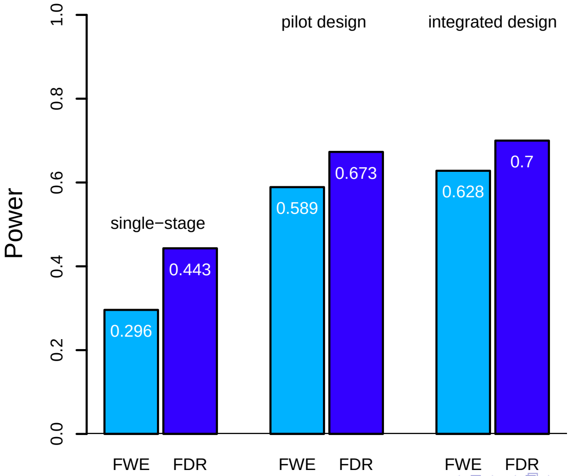
Asymptotic optimal designs

$C = 20000$, $m_1 = 1000$, $\Delta = 0.75$, $\alpha = 0.05$, $\pi_0 = 0.99$, $c_2 = 5$



Asymptotic optimal designs

$C = 20000$, $m_1 = 1000$, $\Delta = 0.75$, $\alpha = 0.05$, $\pi_0 = 0.99$, $c_2 = 15$



Break Even Point in Cost Ratio

If the cost ratio c_2 increases, the power of the two-stage design decreases.

Question

Is there a cost ratio c_2^*, where it does not make sense to apply a two-stage design as compared to the single-stage design?

Integrated design

c_2^* does not exist:
The power of the asymptotic optimal integrated design

- is always larger than of the corresponding single-stage design.
- converges to the power of the single-stage design ($\lim_{c_2 \to \infty} r = 1$)

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Break Even Point: Pilot Design

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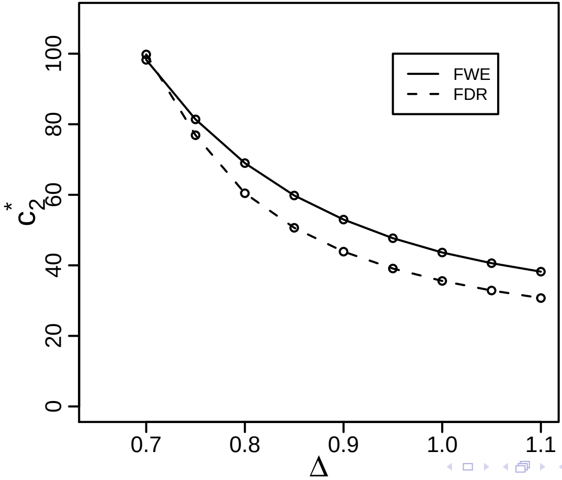

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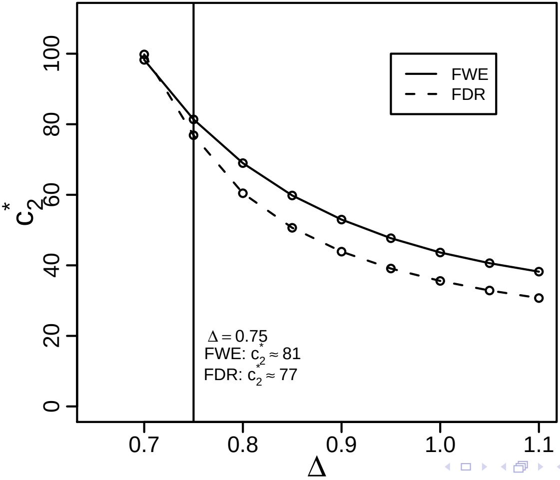

Break Even Point in Cost Ratio

- For the integrated design no such Break Even Point exists.
- For the pilot design such point exist but for small Δ it is large.
- Two-stage designs are a good option to increase the power even if the cost ratio per observation between stages is fairly high.

Impact of Design Misspecifications

Whereas costs are usually known a priori the optimal designs depend on the unknown parameters π_0 and Δ.

Is there an amount of misspecification where it would have been better to use a single-stage design?

Example

- $C = 20000$
- $m_1 = 1000$
- $c_2 = 15$

r and γ_1 planned for the situation:

- $\pi_0 = 0.99$
- $\Delta = 0.75$

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- $c_2 = 15$

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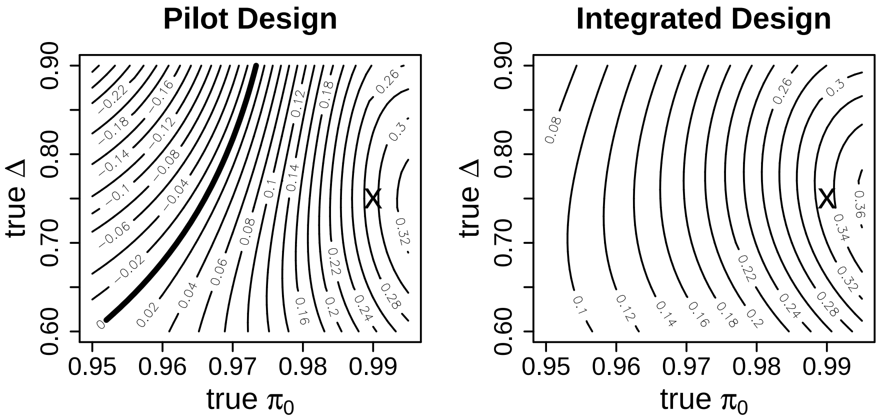
Difference of power values between the two-stage designs and the corresponding single-stage designs (Control of FWE):



X ... point for which two-stage design parameters are optimized

Misspecifications

- The integrated design is more robust against design misspecifications
- if the planned π_0 is larger than the true one:
  loss of power as compared to the single-stage design
- if the planned π_0 is smaller than the true one:
  increase of power as compared to the single-stage design

Scenario 2

The experimenter has two different candidate methods for the measurements from the very beginning:

- low-cost standard method: effect size $= \Delta$
  costs per observation = 1
- high-cost improved method: effect size $= k\Delta$
  costs per observation > 1
- cost ratio between methods $c_2 > 1$
- effect size ratio between methods $k > 1$

Two-Stage Procedures

- first stage: low second stage: low
- first stage: low second stage: high
- first stage: high second stage: high

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Two-Stage Procedures

- first stage: low second stage: low
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Pilot Design controlling the FWE

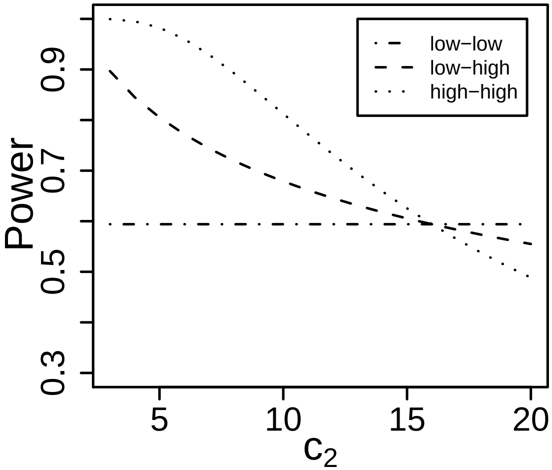
Consider an experiment with

- $\alpha = 0.05$
- $C = 20000$, $m_1 = 1000$
- low-cost method: effect size $\Delta = 0.5$
- high-cost method: $k = 4$ effect size: $0.5 * 4 = 2$

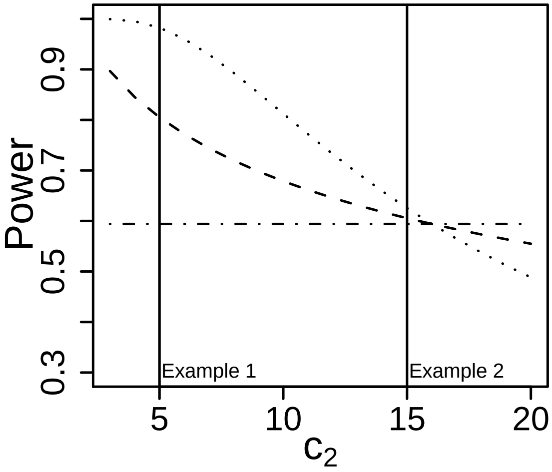
Question

Looking at different values for c_2:
Which of the 3 procedures has the maximal Power?

Common crossing point at $c_2 = k^2 = 16$



Common crossing point at $c_2 = k^2 = 16$



Examples:

Procedure		Example 1: $c_2 = 5$	Example 2: $c_2 = 15$
low-low	Power	0.594	0.594
	n_1	≈ 13	≈ 13
low-high	Power	0.805	0.605
	n_1	≈ 10	≈ 13
high-high	Power	0.983	0.625
	n_1	≈ 3	< 1

Scenario 2

- The low-high procedure may only be preferable if the high-cost method is too expensive to achieve the necessary repetitions at the first stage.
- In our example the high-cost method is not to expensive but highly effective. If we have only double effects for the expensive method than the low-low procedure would be already preferable if the costs are only 4 times larger.
- If the improved method is much more expensive it has to be much more effective to apply a high-high or a low-high procedure.

Conclusions

Different costs in both stages

Two-stage designs are a good option to improve the power even if the cost ratio between stages c_2 is fairly high.

Misspecification

- The integrated design is more robust against misspecification than the pilot design.
- Optimism in the planning phase with regard to the number of true alternatives may help to avoid loss of power.

If two different methods are available

- Depending on c_2 and k it is preferable to run two-stage designs which apply either the low-cost or the high-cost method at both stages.
- Switching from the low-cost to the high-cost method may only be advisable if there is lack of finance so that n_1 for the high-cost method is to small.

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