Quantifying Combination Drug Synergy

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OUTLINE

• “Excess over highest single agent” – a useful first hurdle

• Loewe Synergy

• Loewe Dosewise additivity

• Nonlinear Blending

• Dose Reduction Ratios

• Summary
A Simple (bottom-line) Criterion for Combination Drug Assessment

- “Excess over highest single agent”
  response at (A1,B1) > max {‘response at A1 alone’, ‘response at B1 alone’}

- It is also an FDA criterion for (21 CRF 300.50) for combination drug approval.
Using “Excess over Highest Single Agent” as a Screening Criterion

• “Excess over Highest Single Agent” provides a low-level efficacy hurdle for combination drug screening.

• “Excess over Highest Single Agent” provides the largest window for screening drugs for “dose reduction potential”.

- Note that even with mild antagonism “excess over highest single agent” guarantees some room for “dose reduction potential”, i.e. we can lower both doses of drugs A and B while still maintaining the same response.

- This is useful when one or both of each drug alone has different dose-related side effects.
Testing for Excess over Highest Single Agent

Testing for EOHSA with one fixed dose combination.

\[ H_0 : \mu_{AB} \leq \mu_A \text{ or } \mu_{AB} \leq \mu_B \]
\[ H_1 : \mu_{AB} > \mu_A \text{ and } \mu_{AB} > \mu_B \]

We can test \( H_0 \) vs. \( H_1 \) using the ‘min’ test. This is done by performing two one-sided tests:

\[ H_0^{(A)} : \mu_{AB} \leq \mu_A \text{ vs. } H_1^{(A)} : \mu_{AB} > \mu_A \]
\[ H_0^{(B)} : \mu_{AB} \leq \mu_B \text{ vs. } H_1^{(B)} : \mu_{AB} > \mu_B \]

If we reject both \( H_0^{(A)} \) and \( H_0^{(B)} \), then the min test rejects \( H_0 \).
Simultaneous testing for EOHSA across compound combinations

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<th>Compound B Dose Levels</th>
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- Suppose there is a trend for compound A (for dose levels 0 to 5) at dose level 4 of compound B.
- Suppose also there is a trend for compound B (for dose levels 0 to 4) at dose level 5 of compound A.
- It follows then that the compound combination (5,4) has EOHSA.

C = “combination”
S = “single compound”
V = “vehicle (control)”
Simultaneous testing for EOHSA across compound combinations

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• So intersecting trends can be used test for EOHSA.

• Since there are \( k \) dose levels of each compound there are \( 2(k-1) \) simultaneous trend tests involving exactly \( l \) dose levels.

• At each dose level, \( l \), we can do \( 2(k-1) \) Bonferroni-adjusted trend tests.

• The Tukey step-down trend test can be used as we step from level \( l=(k-1) \) to \( l=1 \).

• The Tukey step-down test requires no adjustment of the (Bonferroni-adjusted) \( \alpha \)-level.

Loewe Synergy
Isobolograms and Combination Index

- The red contour line is a 50% isobologram, i.e. the locus of \((d_1,d_2)\) combination points producing a 50% response.

- Since the contour bows inward we say that we have Loewe synergy.

- Here, the combination index, \(I\), is less than 1 for any \((d_1,d_2)\) combination point on the red dotted line.

- The point \((d_1,d_2)\) has Loewe synergy since \(I\) is less than 1.
What the Combination Index is not Telling You.

(i) Is there enough synergy to overcome the “dilution effect”?

(ii) Will your “synergistic combination” have a better IC50 than you most potent single agent?

(iii) If you have “synergy”, which drug (in combination) provides the best dose-reduction ratio of itself to its (dose-response equivalent) single agent?

\[
\frac{d_1}{ED_{50}^{(1)}} + \frac{d_2}{ED_{50}^{(2)}} = I < 1
\]
Loewe Synergy
Isobolograms and Combination Index

- The blue line is the locus of points such that the total dose equals $A$.

- The $ED_{50}$ for the drug product formed by the 45:55 dose ratio is $A$ since $d_1 + d_2 = A$ at the green point.

- Here the $ED_{50}$ for the combination drug product is greater than that for drug 1 alone despite having Loewe synergy!
Is there a synergy potency?

- If the ED50’s of two compounds are sufficiently different, then the interaction index can produce a troubling result.

- In figures 1 and 2 below both interaction indices are less than one for the combination (green point) on the isobologram.

- However, the ED50 for the combination in Fig. 1 is greater than that for Drug A alone despite a synergistic interaction index!

Fig. 1

Here $I < 1$

Fig. 2

Here $I < 1$
If you have “synergy”, which drug (in combination) provides the best dose-reduction ratio of itself to its a single agent?

• Recall that the Loewe combination index is

\[ I = \frac{d_1}{ED_{50}^{(1)}} + \frac{d_2}{ED_{50}^{(2)}}. \]

• I refer to the individual ratios, \( \frac{d_1}{ED_{50}^{(1)}} \) and \( \frac{d_2}{ED_{50}^{(2)}} \) as dose-reduction ratios.

• So if \( I < 1 \) then \( \frac{d_1}{ED_{50}^{(1)}} \) and \( \frac{d_2}{ED_{50}^{(2)}} \) are both less than 1, \( I=0.7, \) say,

we could have \( \frac{d_1}{ED_{50}^{(1)}} = 0.35 \) and \( \frac{d_2}{ED_{50}^{(2)}} = 0.35 \)

or, we could have \( \frac{d_1}{ED_{50}^{(1)}} = 0.05 \) and \( \frac{d_2}{ED_{50}^{(2)}} = 0.65 \)

• The combination index **confounds** information about the dose ratios!
Plotting Dose Reduction Ratios for Various Drug Combinations

\[ r_2 = \frac{d_2}{ED_{50}^{(2)}} \]

\[ \eta_1 = \frac{d_1}{ED_{50}^{(1)}} \]

Note that the blue dots correspond to combinations with the same interaction index value.
Another Problem with the Interaction Index

Cannot always compute the interaction index!

Monotherapies do not achieve $Y = 50\%$

Yet, excellent synergy exists at a 50:50 ratio!

Acknowledgements to Steve Novick
Problems with Isobologram Approaches to Combination Drug Synergy

• Isobolograms attempt to model the locus of compound-combination points corresponding to a fixed response value, e.g. the 50% (inhibition) response.

• If one (or both) compounds alone do not produce the expected response (e.g. 50% inhibition), then we have may a comparison problem with the combination.

• Furthermore, with isobolograms alone it can be difficult to tell how much they should bow inwards in order to have a synergy of potency (i.e. in order to overcome the “dilution effect” if one of the compounds is not as potent as the other.)

But...suppose we looked at synergy on the response scale, instead if the concentration scale.
Nonlinear Blending

Mixture-Amount Experiments
An alternative approach to analyzing dose-response for combination drugs

• The methodology of “Mixture-amount experiments” has been successfully applied to substance blending experiments in the areas of: fertilizer crop yield studies, pharmaceutical tablet optimization, and detergent formulation.

• A key conceptual difference:
  - Mixture-amount experiments quantify the blending properties of substances for fixed total dose amounts.
  - Isobolograms, on the other hand, attempt to model the locus of compound-combination points corresponding to a fixed response value.

• The isobologram approach can be awkward for situations where the maximum dose-response asymptotes are different or where one or both drugs have no dose response when applied alone (e.g. Augmentin, especially when applied to bacteria that are resistant to amoxicillin.)
The Concept of Nonlinear Blending

- Mixture-amount experiments quantify the blending properties of substances for fixed total dose amounts.

- Consider a fixed total dose, D, of two drug substances (A and B) at varying proportions of drug A

\[\text{total dose} = D\]

**weak nonlinear blending**

\[0\% \text{ drug A} \quad 100\% \text{ drug A}\]

**strong nonlinear blending**

\[0\% \text{ drug A} \quad 100\% \text{ drug A}\]
Nonlinear Blending and Isobolograms

One can show mathematically that strong nonlinear blending $\Leftrightarrow$ synergy of potency for strictly increasing dose-response surfaces.

A “slice” through the response surface for total dose fixed at D

(A’,B’) combination 50% contour line (isobologram)

Synergy on the ray of constant dose ratio. (p% drug A and (1-p)% drug B)
Two examples: Virology and Cancer Chemotherapy

- Virology Example: Drugs AZT and FLG

- The isobologram contour plot for the combination-drug dose response surface.

- There appears to be Loewe synergy here as the interaction index is less than 1 for the 50% (and some other) isobolograms.

- However, the ED50’s for these two drugs are quite different!
Virology Example: Drugs AZT and FLG

- Below, the nonlinear blending plots show that despite a “synergistic” interaction index, blending in FLG with AZT does not help!
In-vitro Cancer chemotherapy example

- Two drugs: 5’FU and a B-Raf inhibitor

- Below, it appears that there is little or no synergy for isobolograms below a 60% response level. (For 5’FU we do not even have an ED70, ED80, etc.)
In-vitro Cancer chemotherapy example

- Below we can see that for some total dose levels, we have strong nonlinear blending, despite little or no evidence of Loewe synergy.
Strong Nonlinear Blending Can Also Be Assessed by Comparing Several Dose Response Profiles for Fixed-Dose Ratios

This perspective is sometimes easier for clients to understand.
Constrained Mixture-Amount Experiments

Line of same total dose

drug 2 dose

drug 1 dose

drug 1 dose response
An Example of Two Drugs with Combination Indices Much Less Than 1 Yet Show No Synergy of Potency

- Drug combination experiment of TMQ (trimetrexate) and AG2034 in the presence of high folic acid.

- Minto-White (parametric) response surface model fitted to data

- Contour plot of dose response surface:
An Example of Two Drugs with Combination Indices Much Less Than 1 Yet Show No Synergy of Potency

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**Key:** Black line = AG2034 alone, Gray Line = TMQ alone, Red line= combination. Note that for TMQ proportions of 0.7, 0.8, and 0.9 the red and gray lines virtually overlap.
An Example of Two Drugs with Combination Indices Much Less Than 1 Yet Show No Synergy of Potency

**Key:** Black line = AG2034 alone, Gray Line = TMQ alone, Red line = combination. Note that for TMQ proportions of 0.7, 0.8, and 0.9 the red and gray lines virtually overlap.
Synergy vs. “Dose Reduction Potential”

• Intuitively, when we think of combination drug synergy we think of two (or more) drugs combining to produce a response greater than what we would expect.

• On the other hand, no such response synergy may exist between two drugs but they may have “dose reduction potential”.

• In other words, we do not expect better responses by combining the drugs but we can nonetheless achieve the same response with smaller amounts of each drug than if we have to use either one alone.

- This is helpful if two drugs have side effects that are different. Or, if one drug has a serious side effect that can be greatly lessened by the addition of a second drug, while still maintaining the same level of efficacy.
Dose Reduction Profile Plots

• Suppose instead we are interested in examining the potential of two drugs to reduce the amount used over each one alone while retaining the same desired level of efficacy.

Here, it is easy to see qualitatively that

\[ d_1 < D_{50}^{(1)} \quad \text{and} \quad d_2 < D_{50}^{(2)}. \]

But it is difficult to determine from the Isobologram the precise values of

\[ \frac{d_1}{D_{50}^{(1)}} \quad \text{and} \quad \frac{d_2}{D_{50}^{(2)}}. \]
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• Chou & Chou (1988) first introduced the notion of looking at the ratios individually.

\[ \frac{d_1}{D_{50}^{(1)}} \quad \text{and} \quad \frac{d_2}{D_{50}^{(2)}}. \]
Dose Reduction Profile Plots

• Suppose instead we are interested in examining the potential of two drugs to reduce the amount used over each one alone while retaining the same desired level of efficacy.

• It is possible to sweep across the isobologram contour line and record each dose ratio

\[ r_1 = \frac{d_1}{D_{50}^{(1)}} \text{ and } r_2 = \frac{d_2}{D_{50}^{(2)}} \]

• Each red arrow is uniquely associated with a specific proportion of drug 1.

• So we should be able to plot \( r_1 \) vs. \( p_1 \) and \( r_2 \) vs. \( p_1 \) for each isobologram contour, where \( p_1 \) is the proportion of drug 1.
Combinations of TMQ and AG2034 in the presence of high folic acid

Nonlinear Blending Plots

Key: Black line = AG2034 alone, Gray Line = TMQ alone, Red line = combination. Note that for TMQ proportions of 0.7, 0.8, and 0.9 the red and gray lines virtually overlap.
Combinations of TMQ and AG2034 in the presence of high folic acid

Dose Reduction Profile Plots

Dose reduction profile plots (high folic acid experiment).
The gray line (TMQ) is the ratio \( r_{TMQ} = \frac{d_{TMQ}}{D_{TMQ}} \),
while the black line (AG2034) is the ratio \( r_{AG2034} = \frac{d_{AG2034}}{D_{AG2034}} \).

• Chou & Chou (1988) and Chou (2006) propose a “dose reduction index” equal to
  \( \frac{D_{TMQ}}{d_{TMQ}} = 1 / r_{TMQ} \) and
  \( \frac{D_{AG2034}}{d_{AG2034}} = 1 / r_{AG2034} \)

• But they only plot \( r_{TMQ}^{-1} \) or \( r_{AG2034}^{-1} \) vs. the corresponding isobol values for
  a fixed proportion of one of the drugs.

• Peterson, J. J. (2010) "A Review of Synergy Concepts of Nonlinear Blending and Dose-Reduction
  Profiles“, Frontiers of Bioscience. S2, 483-503
Some Take-Home Messages for Combination Drug Synergy

- “Excess over highest single agent” (EOHSA) can be a good first hurdle for combination drug screening.

- Nonlinear blending can be applied no matter what the shape of the dose response surface. Issues involving partial inhibitors, potentiation, or coalism pose no problem.
  “Potentiation” = one compound has little or no efficacy by itself.
  “Coalism” = both compounds have little or no efficacy by themselves. (e.g. Augmentin applied to amoxicillin resistant bacteria)

- Strong nonlinear blending implies:
  (i) A synergy of potency (ii) A response synergy (iii) Loewe synergy (if it exists)

- The combination index may be less than 1 but the drugs may not achieve a synergy of potency. This may happen if the compounds have very different relative potencies.

- The combination index confounds information about the ‘dose reduction ratios’. As such, it may be better to examine these ratios individually.
Some References


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