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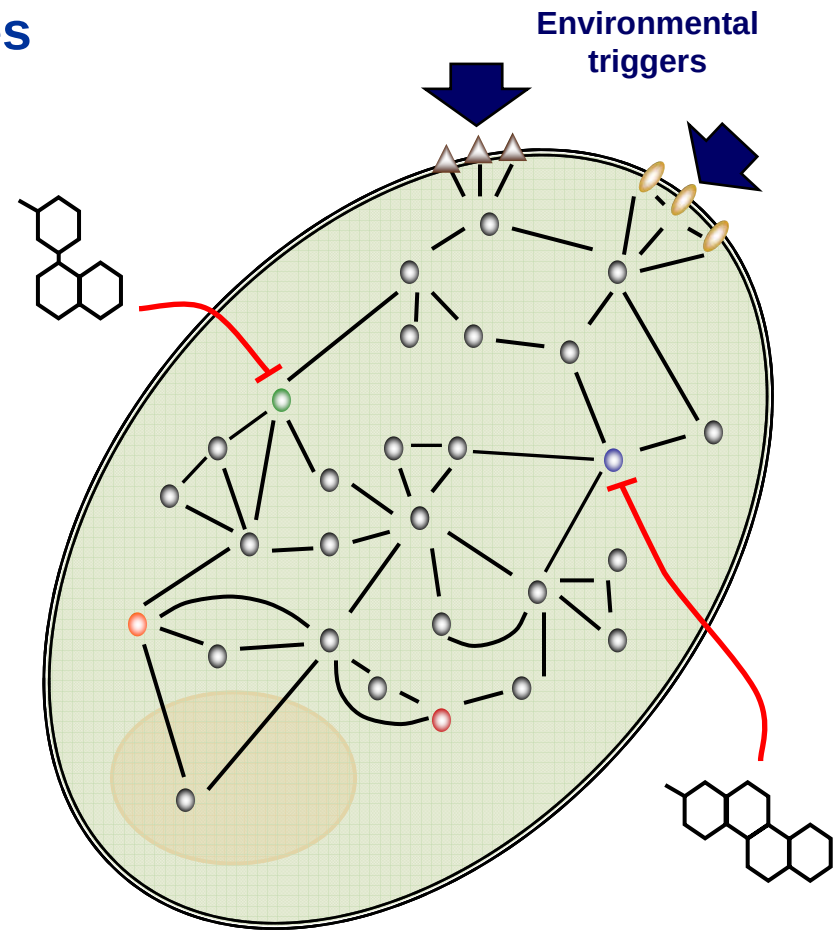
See us. See the future.™

Multi-target Anthrax Antitoxin Therapies
Feb 7, 2007

Why combinations?

- **Limitations of single target therapies**
  - Multifactorial nature of disease
  - Development of resistance

Example: a vital pathway
not stopped by one inhibitor
nor by another alone
but is stopped by both



- **Multi-target Therapies**
  - Utilize cooperative target to create disease modifying effect
  - Higher bar to resistance development

- **Increasingly accepted approach**

>$20B market and growing

(Rebetron®, Augmentin®, Glucovance®, ...)

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Strategies to discover multi-target therapeutics

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Traditional Approach

Intuitive/obvious combinations

Very few combinations can be tested

Unlikely to discover novel multi-target mechanisms

vs.

Systematic Approach

A priori agnostic of mechanism

All combinations can be tested

Can uncover truly novel combination biology

Requires an *in vitro* discovery platform

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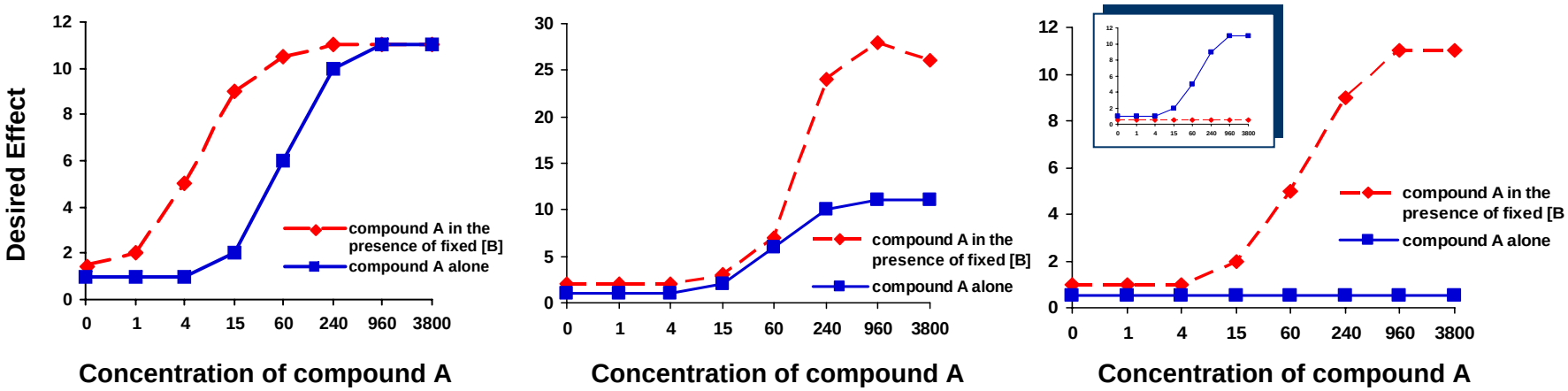
Recognizing Combination Effect

- Observing a shift in compound potency
- Observing a boost in compound maximum effect
- Observing a synthetic effect
- But, effects can happen at any ratio or concentration of component drugs



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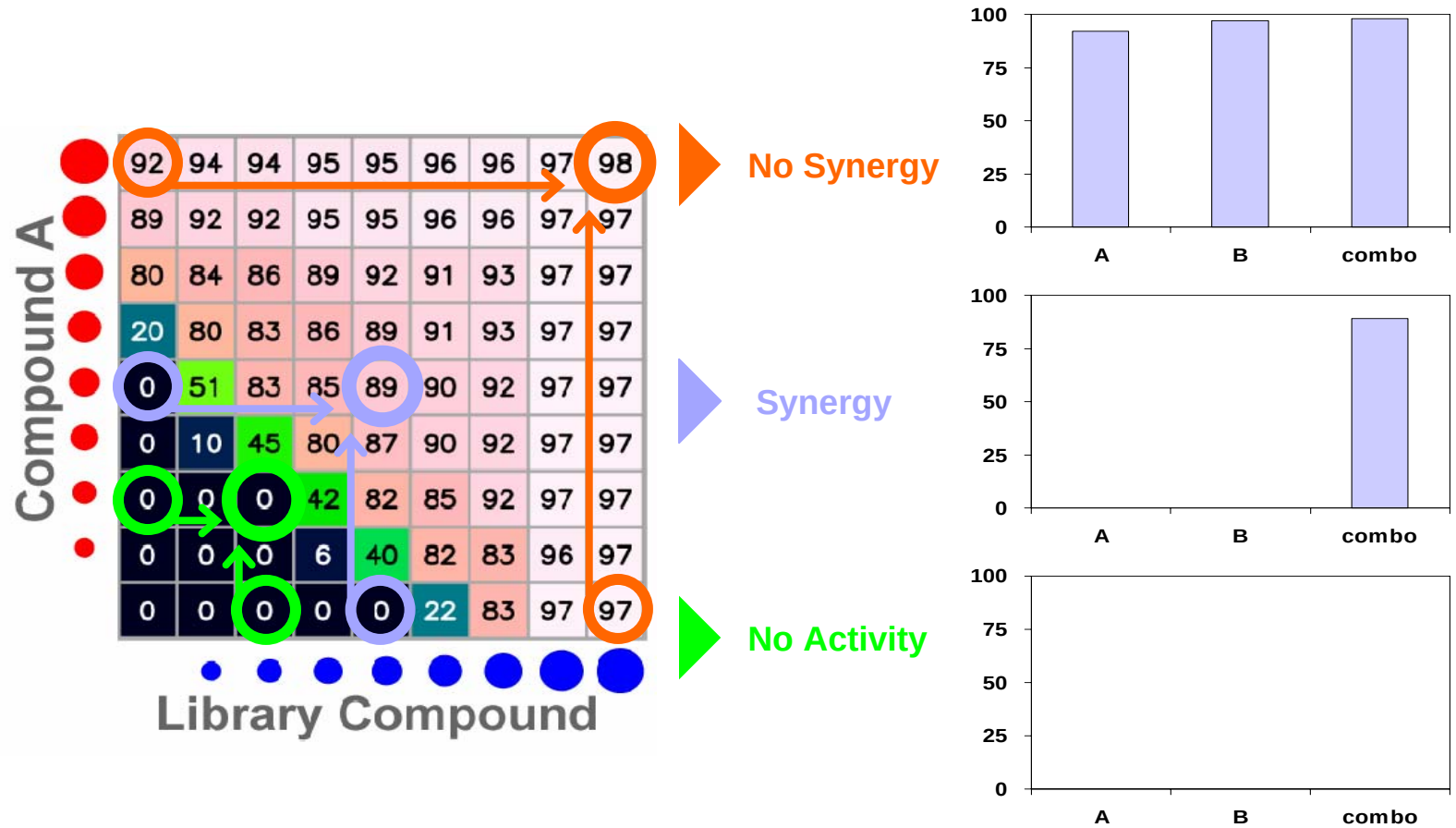
Dose Matrix Screening Unit



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cHTS™ – screening combinations at scale

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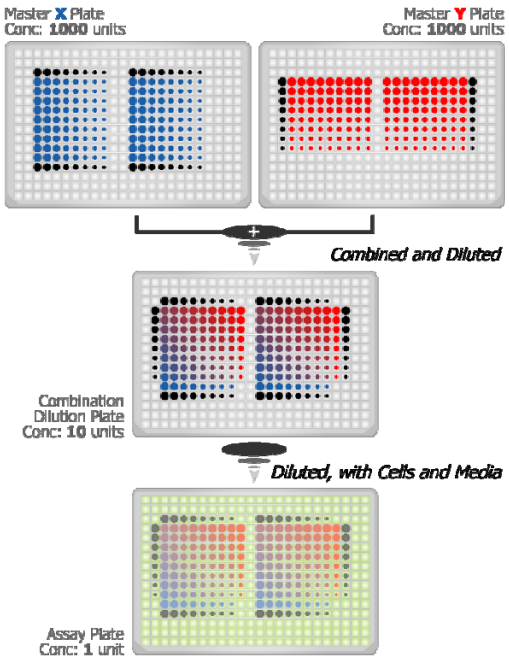
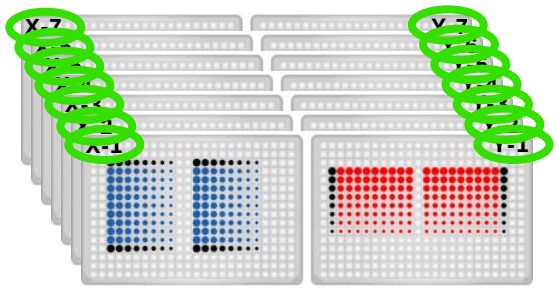
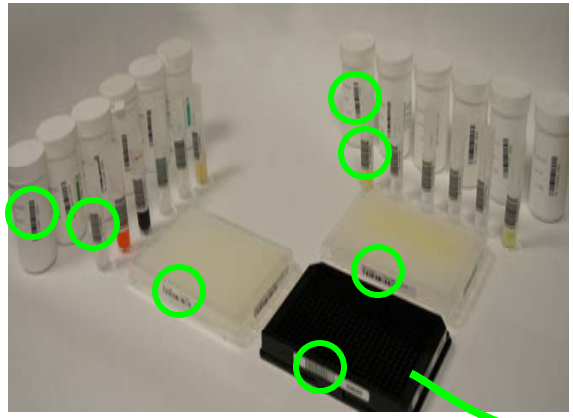
Efficient, proprietary screening methods

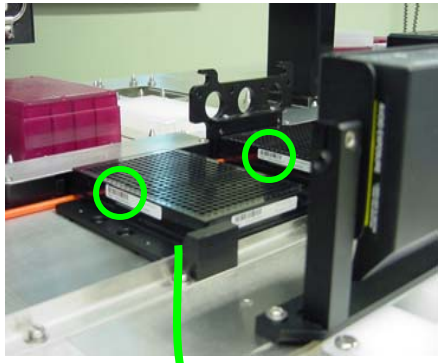


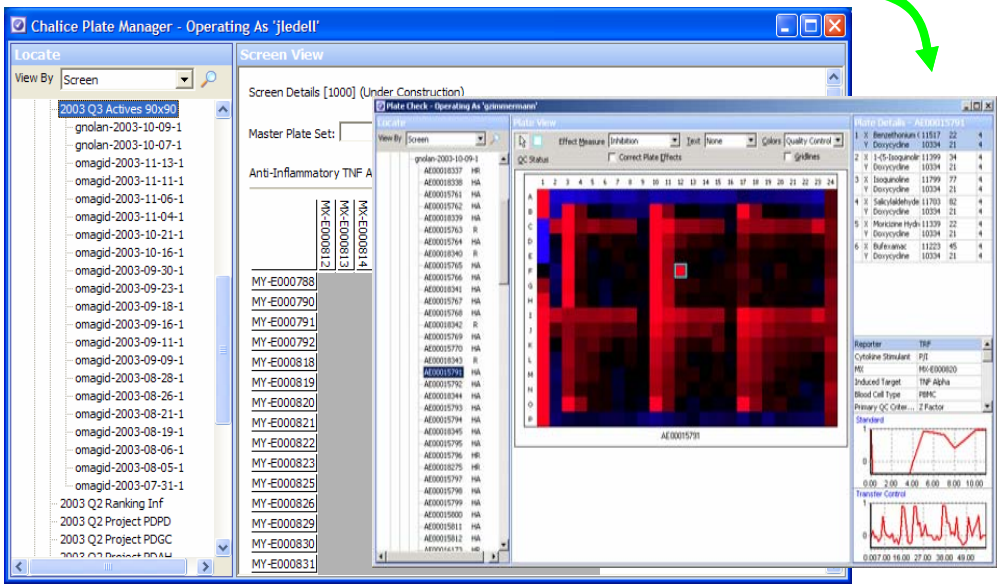


Requiring customized technology

LIMS with automated information tracking







Screening and plate management tools

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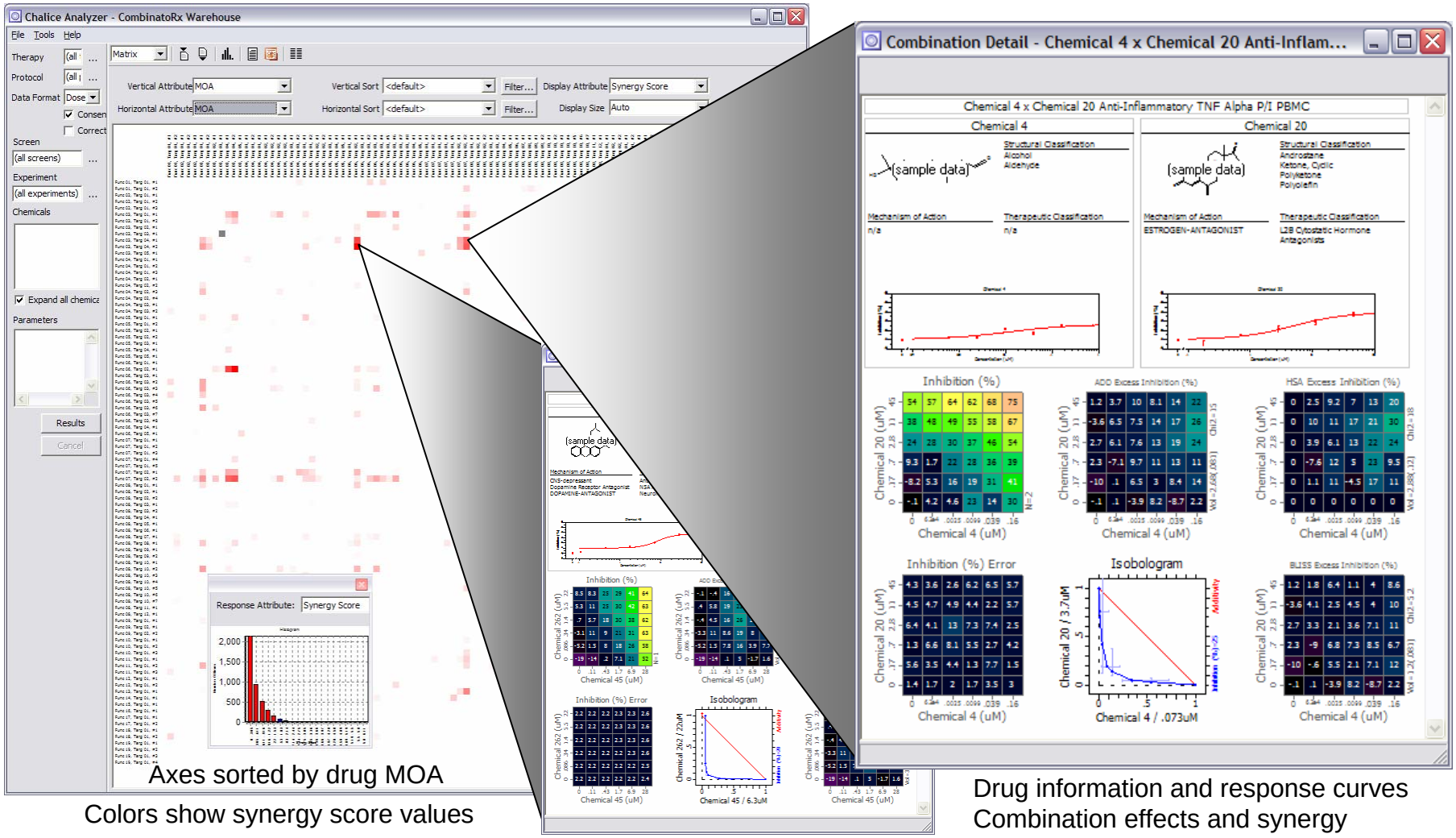
Chalice – selecting hits

Matrix display of whole screen

Expanded views of combination hits



Axes sorted by drug MOA

Colors show synergy score values

Drug information and response curves

Combination effects and synergy

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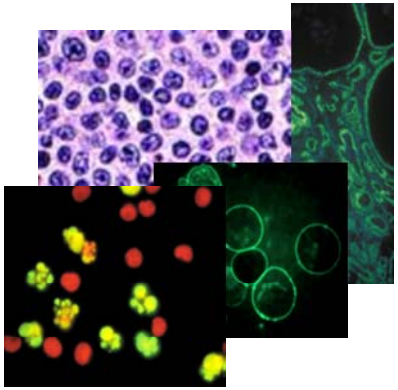
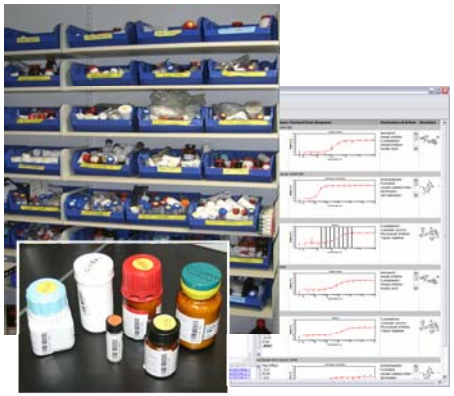
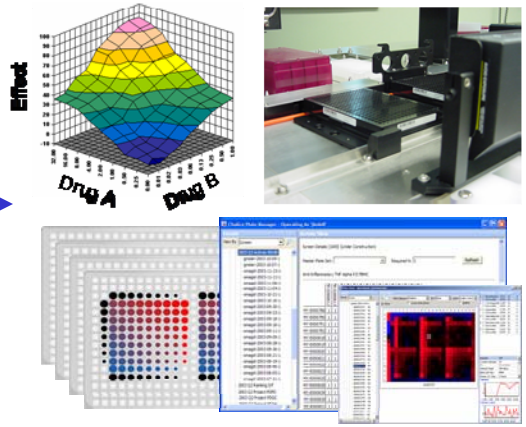
CombinatoRx Discovery Process



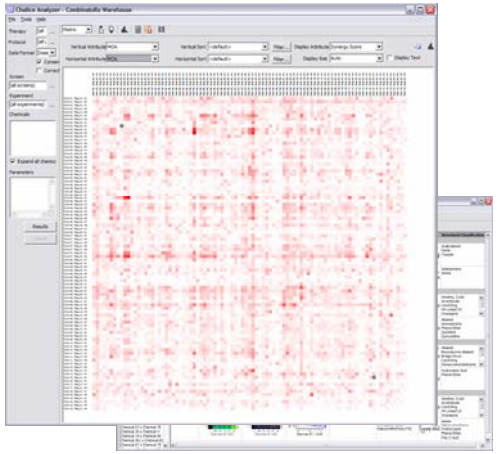
Develop relevant HT assay



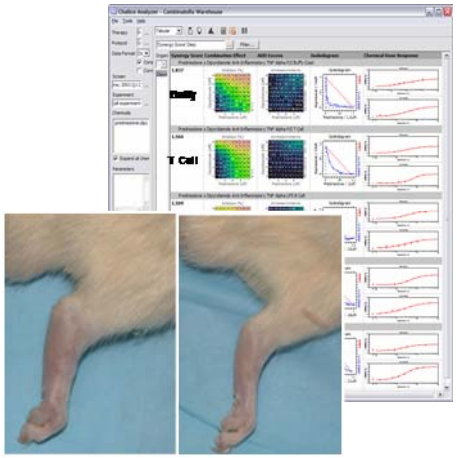
Test library as single agents



Screen pairwise combinations



Select and prioritize hits



Validate in secondary assays and animal tests

Advance leads to clinical trials

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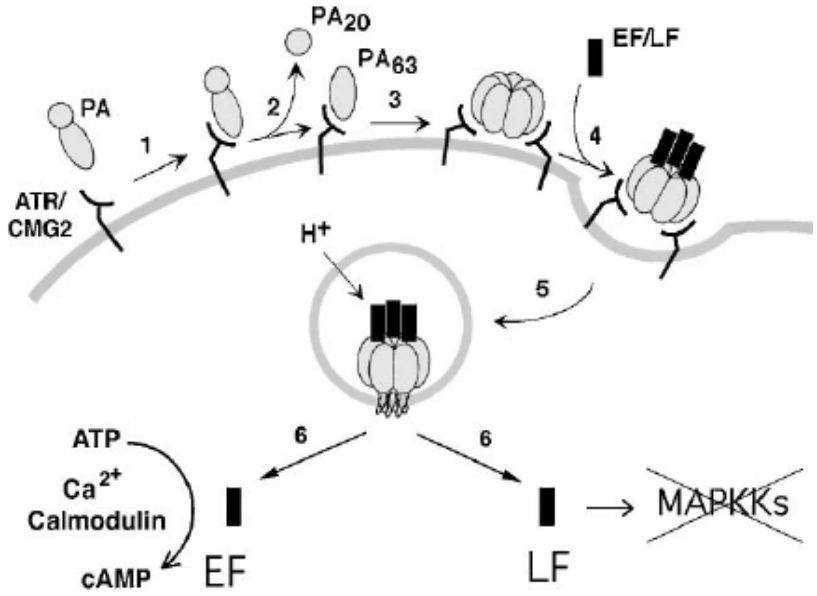
Anthrax Lethal Toxin



Adapted from Collier and Young, Annu. Rev. Cell Dev. Biol, 2003

Lethal toxin is the therapeutic target

Why antitoxin therapy?

- **Narrow timeframe antibiotics effective**
- **Antibiotics not effective against toxin**
- **Compliance and side effects with standard treatment are a concern**
- **Antibiotic or vaccine resistance by "engineered" *B. anthracis***
- **No Anthrax antitoxin product available**

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Anthrax Anti-toxin Discovery Approach

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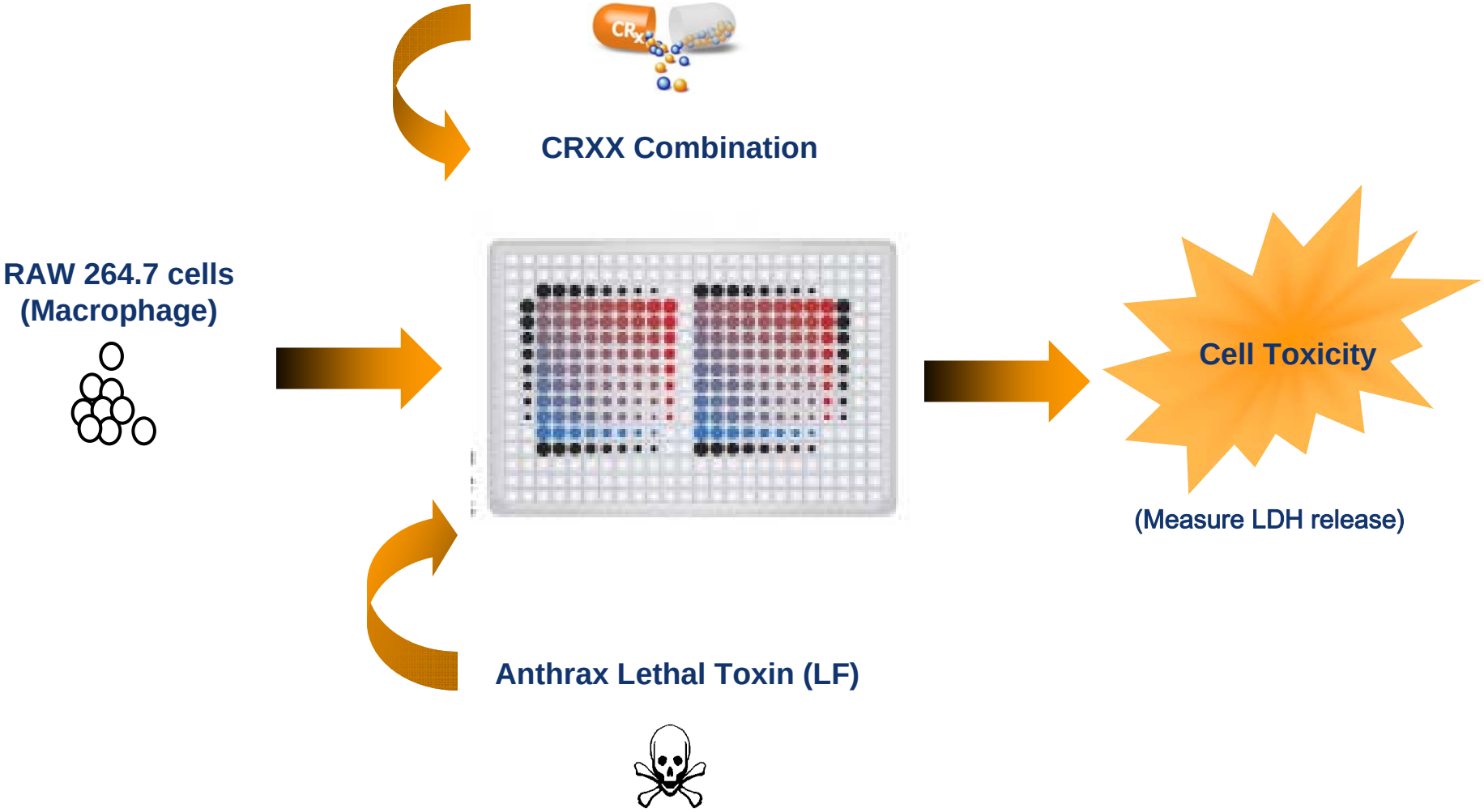
Lethal toxin has profound effects on macrophage cell lines *in vitro*



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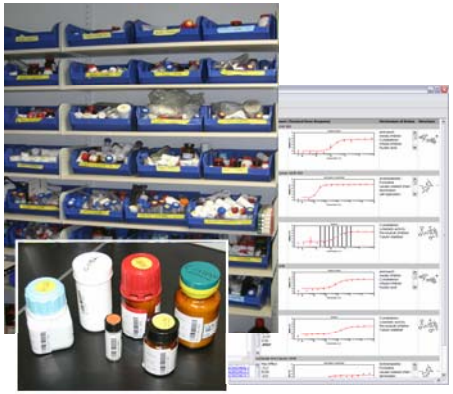
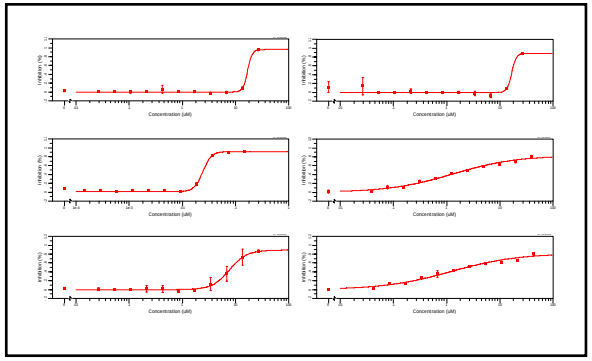
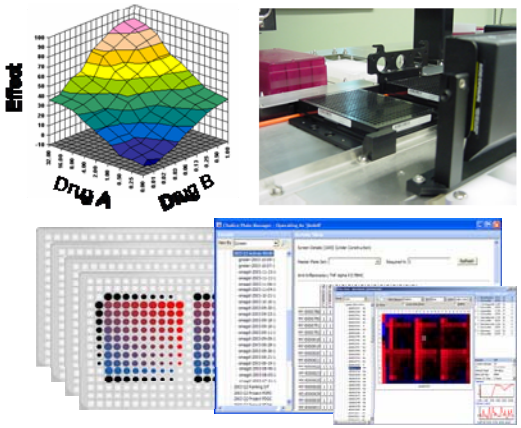
Combination Screen Design



Test library as single agents



Select active single agents



Generate pairwise combinations

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Anti-lethal toxin screen

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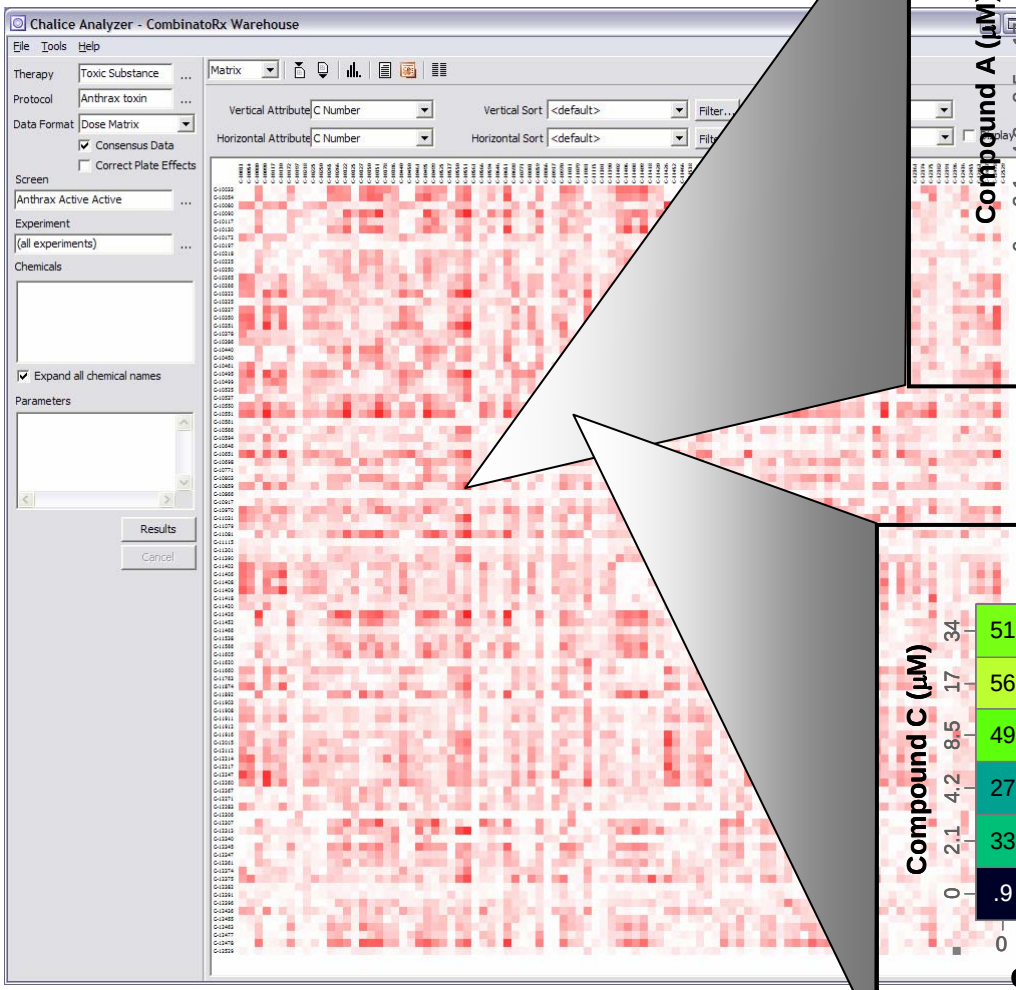
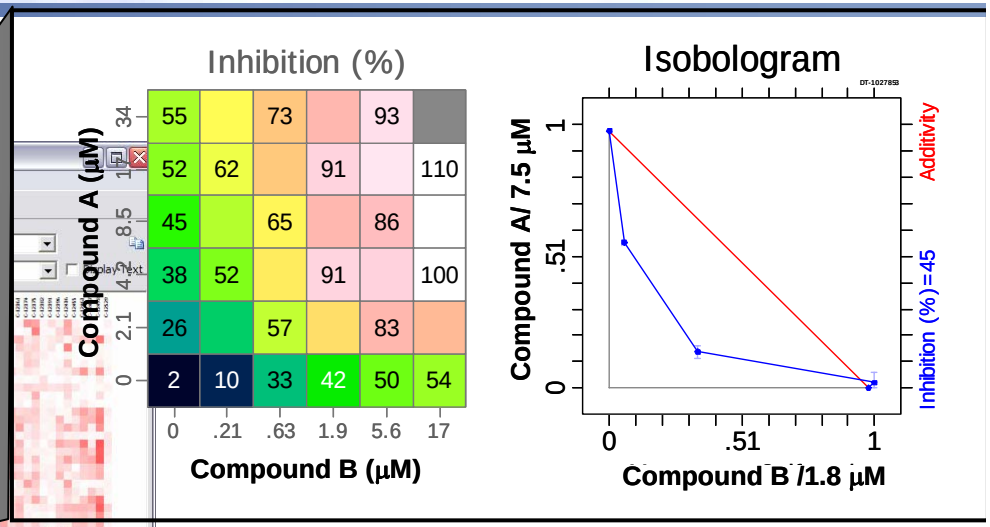
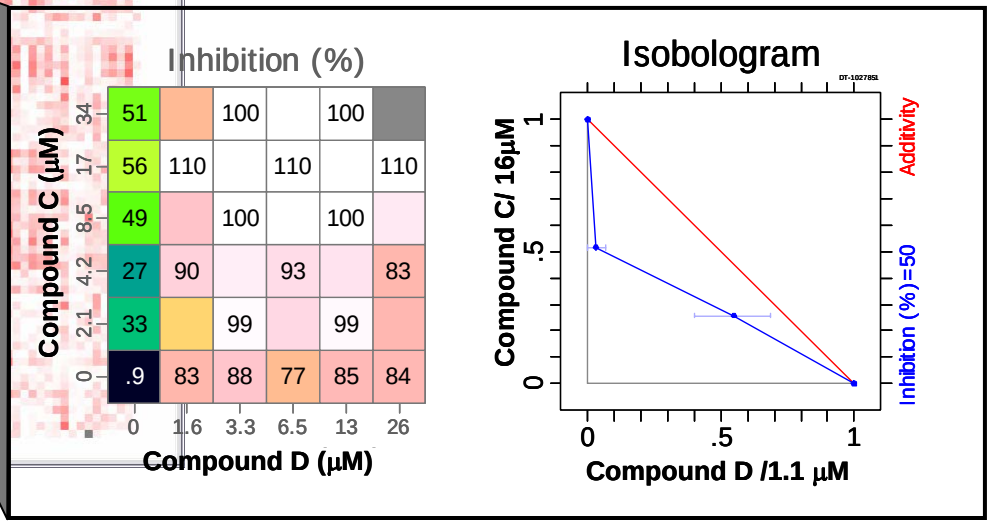
Matrix View of Screen







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Anti-lethal toxin screen

- Identified combinations with antitoxin activity
- Prioritized a subset of combinations for follow up in higher resolution format
- Confirmed activity in alternate cell line
- Prioritized lead combinations for *in vivo* evaluation

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Anti-toxin screen follow up example

Activity in RAW 264.7 cells

Inhibition (%)

Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	50	52	68	70	85
	48	56	72	76	91
3.2	48	51	69	76	87
	35	36	55	55	74
.8	16	21	39	47	62
	4.3	8.6	26	30	38
.2	2.2	4.4	14	15	32
	-2.9	-1.9	8.1	12	16
0	-15	-.4	4.6	3.1	14

84	94	94	99
89	96	94	95
89	89	91	96
75	86	89	89
71	75	79	84
48	64	70	78
38	56	60	67
30	41	46	64
23	37	50	59

N=7-10

ADD Model Inhibition (%)

Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	50	50	50	50	50
	49	49	49	49	49
3.2	46	46	47	47	48
	36	37	37	38	41
.8	16	18	20	24	29
	3.9	6.6	9	13	21
.2	.8	2.5	4.5	8.2	16
	.1	1.5	3.3	6.6	14
0	0	.8	2.1	5.1	12

50	50	50	50	57
49	50	50	57
48	49	50	57
44	47	50	57
37	45	50	57
30	41	50	57
27	40	50	57
26	40	50	57
24	38	50	57

ADD Excess Inhibition (%)

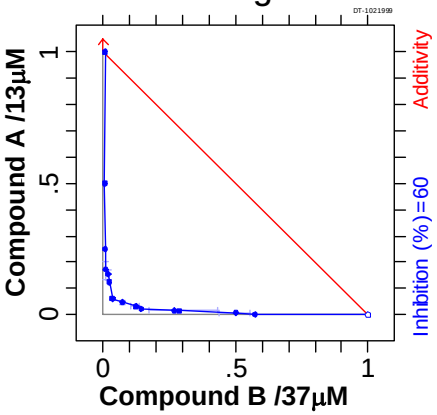
Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	-.1	2.3	18	21	35
	-1	6.6	23	27	42
3.2	1.7	4.8	22	29	39
	-.6	-.3	18	17	33
.8	0	2.9	19	23	33
	.4	1.9	17	17	17
.2	1.4	1.9	9.8	7	16
	-3.1	-3.4	4.8	5.4	1.9
0	-15	-1.2	2.5	-2	2.3

35	44	44	42
40	46	43	37
40	40	41	39
31	39	38	32
35	30	28	27
18	23	20	20
10	17	10	9.7
4.5	1.4	-4.1	7.3
-.5	-1.1	-.8	1.6

Chi2=84

Vol=14(.085)

Isobologram



Activity in J77A4.1 cells

Inhibition (%)

Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	33	40	39	57	63
	33	44	50	60	66
3.2	31	43	46	53	60
	32	33	36	38	50
.8	20	27	35	35	50
	8	13	16	18	35
.2	5.8	6.7	13	17	26
	2.1	1.1	6.9	9.5	20
0	-3.5	-1.4	2.1	4.1	14

65	68	68	78
66	66	71	74
66	67	69	68
55	63	69	64
57	62	62	61
45	50	53	52
36	44	48	48
27	36	43	48
19	30	36	42

N=4

ADD Model Inhibition (%)

Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	33	33	33	33	33
	33	33	33	33	33
3.2	32	32	32	32	32
	29	29	30	30	31
.8	21	22	22	24	26
	9.6	11	13	16	21
.2	3	5.3	7.4	10	16
	.8	2.6	4.5	7.4	14
0	0	1	2.3	5.3	11

33	33	37	41
33	33	37	41
33	33	37	41
32	33	37	41
29	33	37	41
26	32	37	41
24	31	37	41
22	31	37	41
20	30	37	41

ADD Excess Inhibition (%)

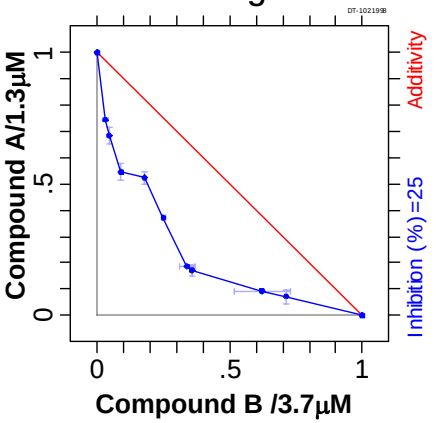
Compound A (µM) \ Compound B (µM)	0	.33	1.3	5.3	21
13	-.4	6.7	5.7	24	30
	.3	12	17	27	33
3.2	-1.1	11	14	21	28
	2.6	4.3	6.5	7.8	19
.8	-.7	5.9	13	11	24
	-1.6	1.3	3.6	1.8	14
.2	2.8	1.4	5.9	6.7	9.9
	1.3	-1.5	2.4	2.1	6.2
0	-3.5	-2.3	-.2	-1.2	3.1

32	34	31	37
32	33	34	32
34	34	32	27
23	30	32	22
27	29	25	20
19	18	16	10
13	13	11	6.3
4.5	5.3	5.6	6.5
-1.5	0	-1.5	1.2

Chi2=180

Vol=11(.026)

Isobologram



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Summary

- **Applied cHTS™ to discover multi-target therapeutics for Anthrax lethal toxin**
- **Identification of synergistic combinations with potent anti-lethal toxin activity**
- **Efficacy of combinations in *in vivo* models and MOA studies are underway**

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Acknowledgements

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  - Shannon Shelton
  - Nicky Sirianni
- **NIAID/NIH**

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