# Augmented Definitive Screening Design Provides Efficient Phase 1 Selection of CAR T-cell and Lymphodepletion Regimen

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## Background & Objective

Lymphodepletion (LD) conditioning regimen prior to CAR T-cell therapy treatment has shown to play a critical role in CAR T-cell therapy success [1-4]. It creates an immunosuppressive environment for T-cell engraftment and expansion. Limited optimization of LD is typically performed while evaluating dose levels of CAR T-cells in Phase 1/2, setting the stage for underperformance of promising emerging interventions and regulatory scrutiny (i.e. Project Optimus) especially in the allogeneic CAR T-cell therapy space when adding alemtuzumab (ALEM) to the standard LD regimen of cylophosphamide (CY) and fludarabine (FL) to overcome potential alloimmune rejections.

## Methods

Previously, we have commented that combination drug trials could be improved with the use of Definitive Screening Designs (DSD) [5-6]. Use of this design in the context of CAR-T therapy was studied by simulation, selecting single CAR T-cell dose levels of 50, 100, 150 million cells, FL dose levels of 20, 30, 40 mg/m2 for three days, CY dose levels of 300, 400, 500 mg/m2 for three days, and ALEM dose levels of 0, 20, 30 mg for three days. A full factorial design would require $3^4$=81 cohorts, which is impractical for a Phase 1/2 study. A DSD was used to augment a traditional monotherapy design (Mono; varying only CAR T-cell dose levels over n=3 cohorts) for a total of n=11 cohorts; one cohort of DSD and Mono are identical.

### Assumptions

Probability of CAR-T yielding positive patient benefit-risk [50%, 100%] is dependent on CAR-T dose and the use of LD components. Reduction in logit(probability) [0, -1] dependent on LD component dose.

Three dose levels for each component, higher and lower intensity than the standard regimen (ie. the centerpoint):

- CAR-T 50, 100, 150 million cells
- Flu 20, 30, 40 mg/m2 x3
- Cy 300, 400, 500 mg/m2 x3
- Alem 0, 20, 40 mg x3

Alemtuzumab is appropriate for allogeneic CAR-T; bendamustin (60 mg x3) could be substituted, or this factor simply dropped.

### Trial Design

Design options:

- **Monotherapy**: CAR T 50, 100, 150 million cells and LD components all at one value. This reflects current practice.
- **DSD**: 9 distinct component combinations

| Design | CART, million cells | Flu, mg/m^2 x3 | Cy, mg/m^2 x3 | Alem, mg x3 |
|---|---|---|---|---|
| Monotherapy | 50 | 30 | 400 | 20 |
| Monotherapy | 100 | 30 | 400 | 20 |
| Monotherapy | 150 | 30 | 400 | 20 |
| DSD | 100 | 40 | 300 | 0 |
| DSD | 100 | 20 | 500 | 40 |
| DSD | 50 | 30 | 300 | 40 |
| DSD | 150 | 30 | 500 | 0 |
| DSD | 50 | 20 | 400 | 0 |
| DSD | 150 | 40 | 400 | 40 |
| DSD | 50 | 40 | 500 | 20 |
| DSD | 150 | 20 | 300 | 20 |
| DSD | 100 | 30 | 400 | 20 |

Two new treatment combinations suggested from analysis of the observed benefit-risk:

- **Univariate**: component dose demonstrating best mean benefit-risk.
- **Multivariate**: component doses estimated to have best mean benefit-risk.

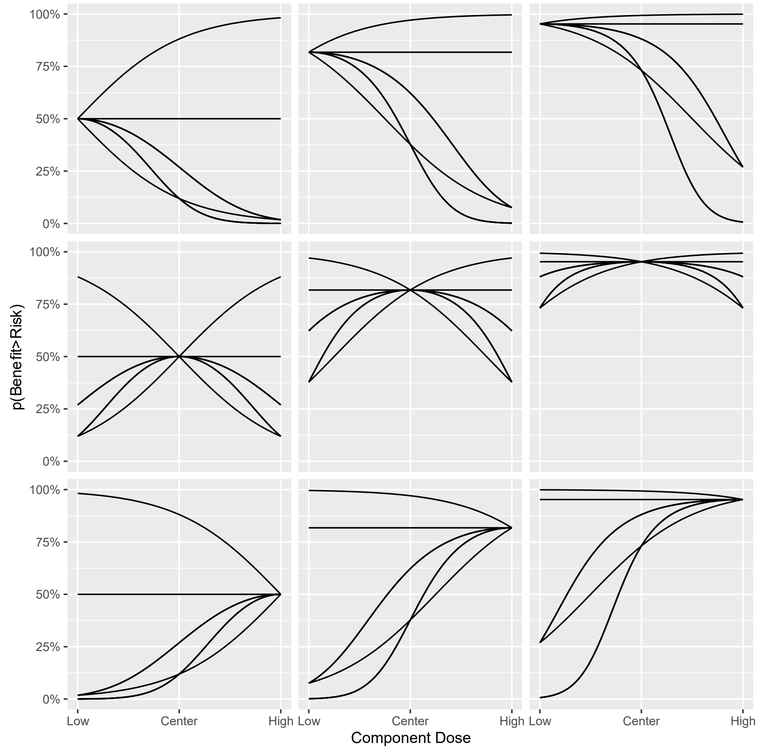
Response surface model: linear one-factors, linear two-way interactions and pure quadratic model for logit(p(B-R))

Each recommended regimen (Monotherapy, DSD, Univariate, Multivariate) contrasted against unknown, **Optimal** benefit-risk by odds-ratio (OR). N=1000 simulation studies were conducted.

## Flexible Dose Response Models

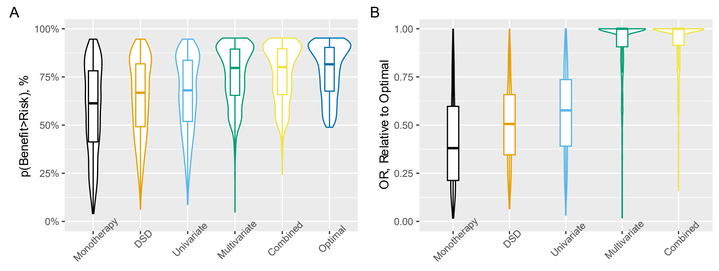
Dose response models:

- In logit space: Intercept, Linear, Quadratic, Linear+Quadratic
- A linear model in logit space corresponds to a Hill model in probability space.
- A quadratic model in logit space corresponds to a U-shaped model in probability space.




## Results

Simulations of the probability of a patient receiving a favorable benefit-risk outcome, p(B-R), were conducted across plausible ranges of CAR T-cell + LD dose-response relationships to compare each approach to the true but unknown optimal p(B-R).




Each design (Monotherapy, DSD, Univariate, Multivariate) identified regimens that approached the Optimal benefit-risk

**Panel A** shows the best benefit-risk that could be identified by each design.

- **Monotherapy**: best of three regimens shown, but in many cases the optimal LD was not the centerpoint, so this approach is quite vulnerable to suboptimal LD choices.
- **DSD**: best of eight regimens shown, and there are more opportunities to vary LD and locate improvements.
- **Univariate**: one regimen shown, and the balanced nature of the design allows reasonable accuracy.
- **Multivariate**: one regimen shown, and the linear modeling approach allows reasonable accuracy.
- **Combined**: best benefit-risk of Monotherapy, DSD, Univariate, Multivariate gained from the study.

**Panel B** shows the best benefit-risk that could be identified by each design, given as an odds-ratio.

- OR=p(<method>)/p(Optimal)

| | Monotherapy | DSD | Univariate | Multivariate | Combined | Optimal |
|---|---|---|---|---|---|---|
| **p(Benefit>Risk), %** | | | | | | |
| Mean (SD) | 58.2 (23.0) | 64.2 (19.9) | 66.1 (19.4) | 76.4 (15.0) | 76.8 (14.6) | 78.3 (13.6) |
| Median [Min, Max] | 61.3 [4.04, 94.6] | 66.8 [6.26, 94.6] | 68.0 [8.78, 94.6] | 79.7 [4.80, 95.2] | 80.1 [24.3, 95.2] | 81.6 [48.9, 95.2] |
| **OR, Relative to Optimal** | | | | | | |
| Mean (SD) | 0.419 (0.242) | 0.509 (0.210) | 0.562 (0.224) | 0.917 (0.160) | 0.928 (0.137) | 1.00 (0) |
| Median [Min, Max] | 0.381 [0.0170, 0.998] | 0.506 [0.0661, 0.998] | 0.576 [0.0326, 1.00] | 1.00 [0.0175, 1.00] | 1.00 [0.160, 1.00] | 1.00 [1.00, 1.00] |

The Monotherapy, Combined and (true) Optimal p(B-R) estimates were mean 58.2% (standard deviation (sd) 23%), 76.8% (14.6%), and 78.3% (13.6%), respectively. The odds ratio of Monotherapy and Combined to true Optimal was mean 0.419 (0.242) and 0.928 (0.137), respectively.

This translates to 2.37-fold higher odds of a patient achieving favorable benefit-risk profiles using the augmented DSD approach versus traditional designs.

## Conclusions

Optimizing CAR T-cell and LD dose regimens in Phase 1/2 clinical trials is paramount to the success and optimal benefit-risk outcome of CAR T-cell therapies. Beyond optimizing patient benefit-risk ratio and meeting Project Optimus guidance, drug development timelines can be accelerated: a confirmatory trial with one cohort is sufficient following a well-designed Phase 1/2 trial design.

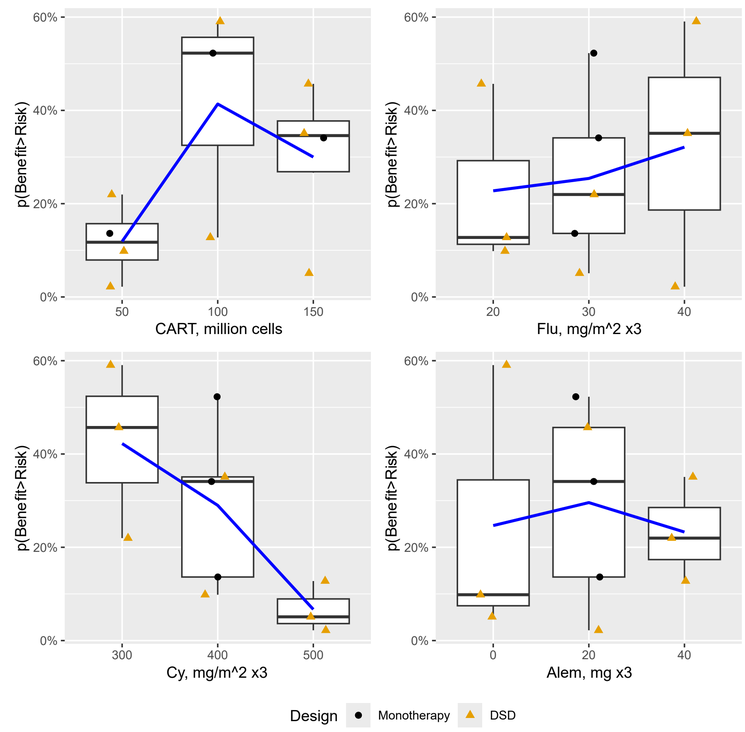
## Example Case: Best Observed Regimen

Three cohorts (1x Monotherapy, 1x DSD) show p(Benefit>Risk) > 50%. Optimal is 68.5%.

| Design | CART, million cells | Flu, mg/m^2 x3 | Cy, mg/m^2 x3 | Alem, mg x3 | p(Benefit>Risk), % | OR, Relative to Optimal |
|---|---|---|---|---|---|---|
| Monotherapy | 50 | 30 | 400 | 20 | 0.1363 | 0.07253 |
| **Monotherapy** | **100** | **30** | **400** | **20** | **0.5228** | **0.5035** |
| Monotherapy | 150 | 30 | 400 | 20 | 0.3411 | 0.2379 |
| **DSD** | **100** | **40** | **300** | **0** | **0.5906** | **0.6631** |
| DSD | 100 | 20 | 500 | 40 | 0.1276 | 0.06723 |
| DSD | 50 | 30 | 300 | 40 | 0.2197 | 0.1294 |
| DSD | 150 | 30 | 500 | 0 | 0.0509 | 0.02465 |
| DSD | 50 | 20 | 400 | 0 | 0.09831 | 0.05011 |
| DSD | 150 | 40 | 400 | 40 | 0.3509 | 0.2485 |
| DSD | 50 | 40 | 500 | 20 | 0.02202 | 0.01035 |
| DSD | 150 | 20 | 300 | 20 | 0.4569 | 0.3867 |
| DSD | 100 | 30 | 400 | 20 | 0.5228 | 0.5035 |
| **Univariate** | **100** | **40** | **300** | **20** | **0.6607** | **0.8951** |
| **Multivariate** | **112.5** | **40** | **325** | **30** | **0.6837** | **0.9935** |
| *Combined* | *112.5* | *40* | *325* | *30* | *0.6837* | *0.9935* |
| *Optimal* | *112.5* | *40* | *300* | *30* | *0.6851* | *1* |

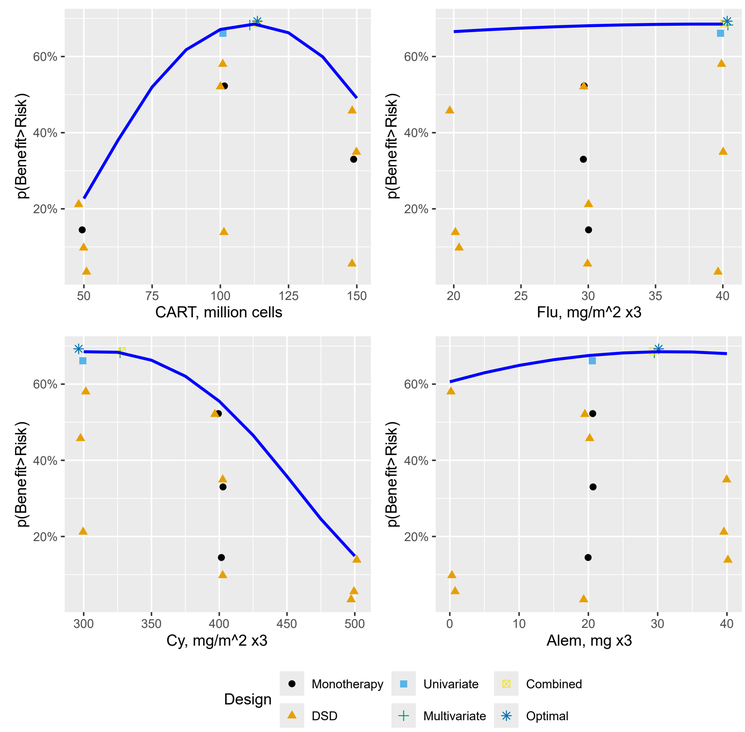
## Example Case: Best Estimated Univariate Regimen

As the design is balanced in the components, effect sizes can be calculated in a univariate manner. Blue lines show the mean of p(Benefit>Risk) the univariate dose-response.




## Example Case: Best Estimated Multivariate Regimen

Linear model of p(Benefit>Risk) constructed, allowing for linear and quadratic effects of each component and linear interaction between components. Blue lines show the estimated multivariate dose-response for each component with other components set to their optimal values.

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