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PLATFORM APPROACH FOR IDENTIFYING THE MOST FUNCTIONAL AND DEVELOPABLE BISPECIFIC T-CELL ENGAGER

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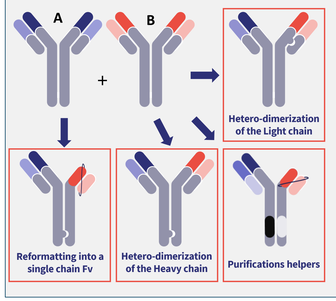
aaps PharmSci 360

PURPOSE

What are the main considerations when designing a bispecific antibody?



Bispecific antibodies are complex and often difficult to design

Multiple technologies have been developed that can aid in the formation of bispecifics, including:

- Technologies around **heavy chain hetero-dimerization**, such as 'Knob into hole'
- Technologies around **light chain hetero-dimerization**, such as CrossMAb or reformatting to scFvs

Other consideration include:

- **Proximity** and **arrangement** of the functional arms
- The **stoichiometry** (1:1, 2:1, 2:2 etc) required for optimal function

OBJECTIVE

The purpose of this study was to evaluate a platform for optimal bispecific design and selection.
Two functionalities were evaluated:
Cancer target – anti CD19, T cell recruiter – anti-CD3

Attribute	Aim	Attribute	Aim	Attribute	Aim
Purity	High, after simple purification	Target Affinity	High affinity to both targets	Biological function	T cell recruitment and killing
Yield	High from standard CHO expression	Stability	Limited chain mispairing	Safety	Limited cytokine activation

METHOD

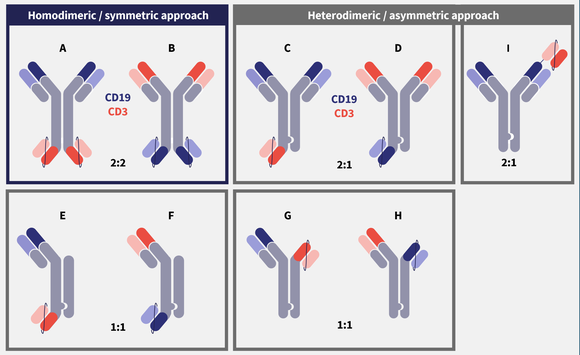
Process flow

Designs
↓
Expression in ExpiCHO cells (7-10 days)
↓
Affinity purification using Protein A
↓
QC and testing – aSEC, CE-SDS, Mass spec
↓
SPR
↓
T cell activity assay
↓
Cytokine storm screening
↓
Final selection

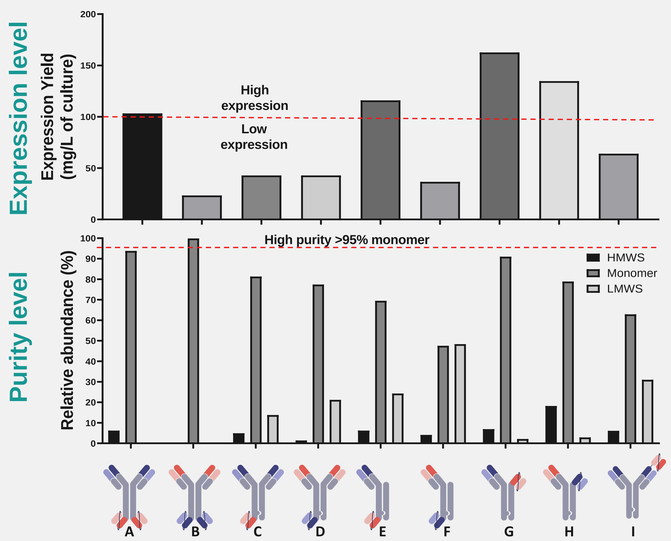
Designs

Nine bispecific constructs were designed, focused around both homo- and hetero-dimeric approaches, including:

- **Inclusion of an Fc** – extend half-life compared to fragment-based TCEs
- **Fc silenced** to improve safety profile
- **Multiple avidities** e.g. 2:2, 2:1, 1:2 and 1:1
- **Alternative spacing** of arms both distal and proximal to each other
- **Alternative formats**: scFv vs Fab



RESULTS – Expression and Characterization



Analysis

All samples were analysed by CE-SDS, Analytical SEC. The data for Design G is shown below, including additional intact and reduced mass spectrometry analysis.

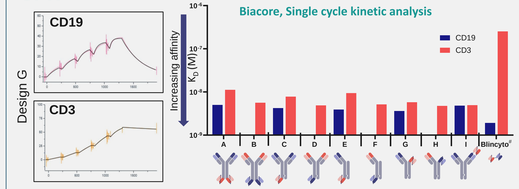
Mass spectrometry

RESULTS - Functionality

A successful T cell engager should have affinity to both targets, CD19 and CD3. IT should be able to induce a strong T cell response and show active T cell killing through recruitment. Most importantly, it should also be safe.

Herein we triage our nine starting designs via SPR binding, T cell activation, T cell recruitment and killing and a cytokine release assay to determine the optimal T cell engager designs for further studies.

SPR

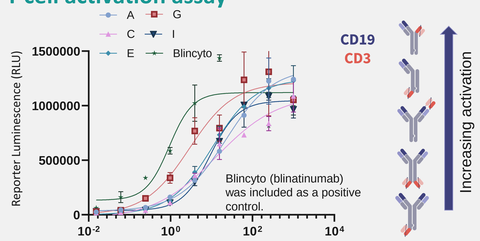


SPR analysis shows a clear distinction between designs. All anti-CD19 scFv designs showed no detectable binding at the concentration tested (*), suggesting reformatting to scFv has been detrimental.

All other designs showed strong binding to both antigens. All anti-CD19 scFv designs were eliminated from further study.

Dreier, T. et al. Extremely potent, rapid and costimulation in dependent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. Int. J. Cancer 100, 690–697 (2002).

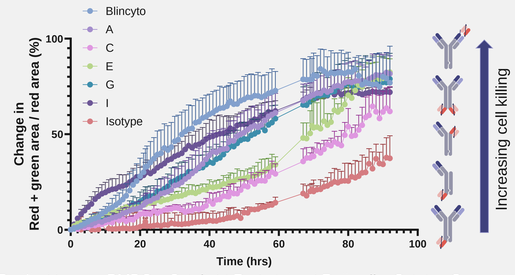
T cell activation assay



A T cell Activation Bioassay using Raji (CD19+) target cells and TCR/CD3 effector cells (NFAT) was used to measure the activity of all bispecific designs.

Of the remaining designs, several showed strong activation with those designs having a closer proximity of its two arms typically showing a higher activation.

Cancer Cell Killing via Incucyte®

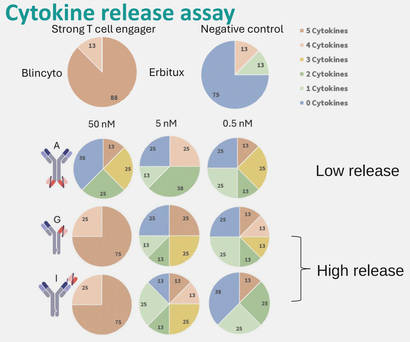


Purified human PMBCs, Cytolight Red-stained Raji cells, Cytotox Green reagent (indicator for dead cells) and test bispecifics were incubated for 96 hours and monitored using an Incucyte® live cell imaging system. As T cells are recruited and cancer cells killed, the red and green stains mix, the production of a yellow colour is monitored and is shown in the figure above.

Of the remaining designs, three show a strong ability to recruit and kill cancer cells (Designs I, A and G).

Two designs showed limited recruitment activity (design E and C).

Cytokine release assay



The stimulation of cytokines after incubation with human blood provides an indication of potential safety concerns. Interestingly out of the 3 designs with the best killing potential, only design A showed a low toxicity profile.

CONCLUSION

Attribute	Aim	Attribute	Aim	Attribute	Aim
Purity	High level of purity from a single step ~95% monomer	Target Affinity	High affinity observed in SPR for both targets	Biological function	Strong T cell recruitment and killing
Yield	Standard level of expression of around 100 mg/Litre from transient transfection	Stability	Symmetrical design limits chain mispairing and provides an easier downstream development route	Safety	Potential low risk of cytokine storm

- From in-vitro analysis, Design A is a strong T cell engager candidate for further in-vivo studies, with design I and G being good backups
- Key learning from this project include the importance of gaining high purity from simple purifications and the potential problems with reformatting into a single chain Fv format
- It is difficult to predict the optimal bispecific format, meaning that **screening of multiple designs** should be investigated

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