Aptevo Therapeutics

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# Screening Next Generation ADAPTIR™ Bispecific Proteins for Manufacturability and Function

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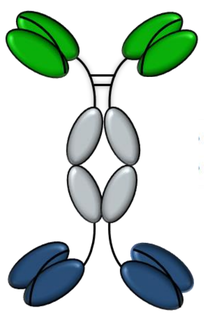
## Introduction

The ADAPTIR™ bispecific protein scaffold has been developed in parallel with a robust screening methodology to identify lead candidates with the desired functional and manufacturability characteristics. Molecules including monospecifics, bispecifics and cytokine fusion proteins with a variety of mechanisms of action have been produced. The platform has resulted in several stable candidates that are approaching or are currently in human clinical trials. Examples include APVO-210 (anti-CD86 x IL10 fusion), APVO-436 (anti-CD123 x anti-CD3) and ALG.APV-527 (anti-4-1BB x anti-5T4), which is being codeveloped with Alligator Biosciences.

Here we highlight examples of manufacturability data collected while screening new binding domains and during their subsequent optimization to address potential liabilities. This includes evaluations of colloidal and conformational stability and post-translational modifications. Additionally, we show the impact of thermal stabilization and optimization of the ADAPTIR scaffold on mouse pharmacokinetics and biodistribution.

## ADAPTIR Bispecific T-Cell Engagers




ADAPTIR bispecific therapeutics contain two sets of binding domains linked to an immunoglobulin Fc domain, which enables an antibody-like half-life. To avoid interactions with other components of the immune system that could lead to non-specific T-cell activation, the Fc region can be engineered to minimize complement fixation and Fcγ receptor binding.

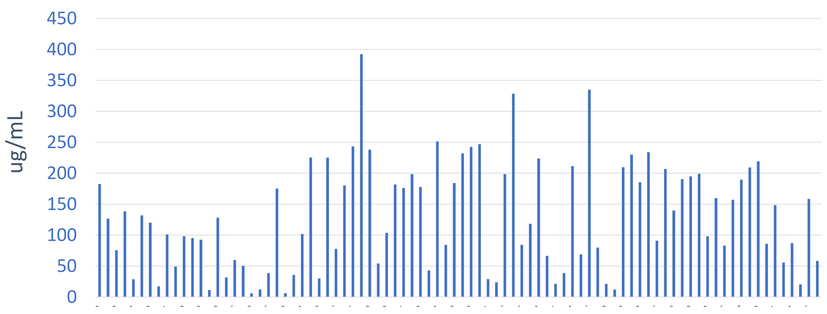
ADAPTIR bispecific proteins have been generated against several tumor-associated antigens and immune effector molecules, including CD3 and 4-1BB.

## Candidates are Screened in Several Orthogonal Assays to Assess Manufacturability

| Category | Assays |
|---|---|
| Thermostability | DSC (Tm) |
| | DSF (Tm, $T_{agg}$) |
| Solubility | High-salt solubility |
| | Protein concentration |
| Process compatibility | Process intermediate stability (pH, salt, temperature and time) |
| | Shear stress stability |
| Storage stability | Stability at multiple pH, temp, [protein] and platform formulation conditions |
| Sequence liabilities | PTM prediction and evaluation by forced degradation and LC-MS |
| | Spatial Aggregation Propensity (SAP) Analyses |
| Specificity, biological stability | Target binding affinity |
| | Non-specific binding screens |
| | Serum stability (binding and function) |
| | Protease susceptibility |

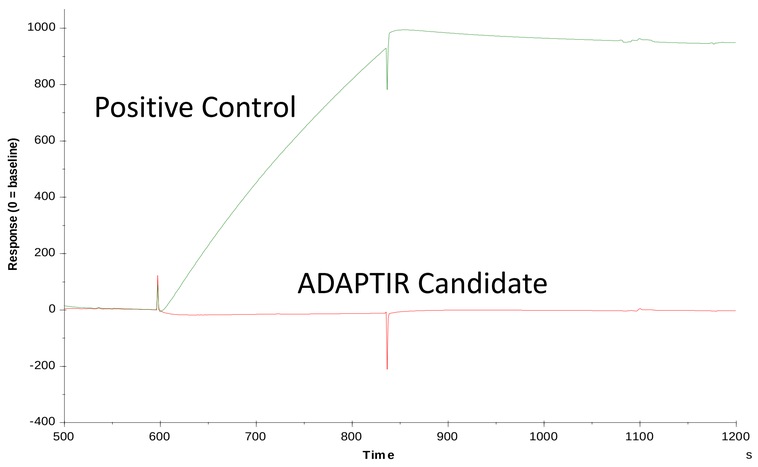
Bispecific candidates are screened in a standardized panel of assays that can be performed with relatively small quantities of protein. These assays probe different aspects of protein stability under conditions during manufacturing, dosing, or *in vivo*.

## ADAPTIR Transient Expression Evaluation



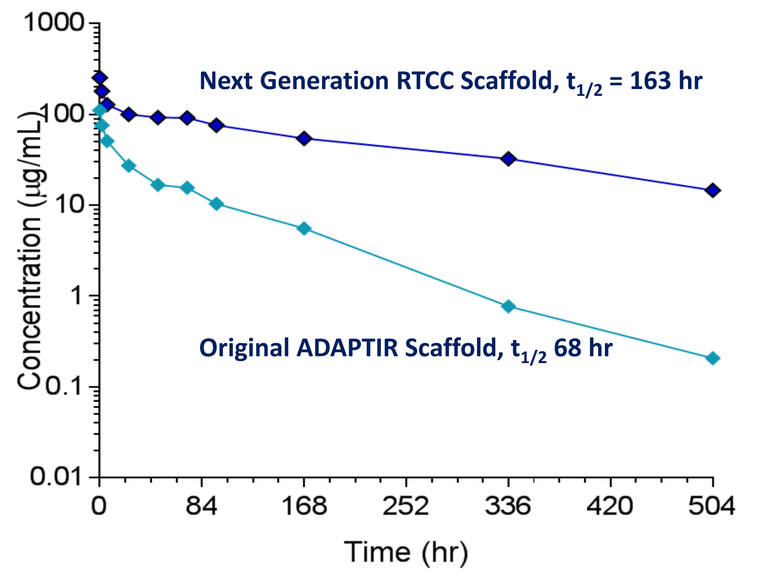

Transient expression levels are considered during the initial screening of new bispecifics to assure that subsequent stable cell expression levels can meet clinical and commercial needs.

## Candidates are Screened for Nonspecific Binding to Serum and Cell Surface Proteins



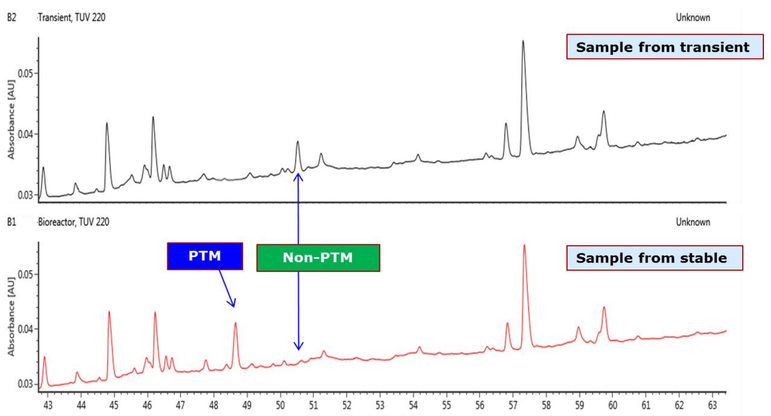

Due to the potency of bispecific T-cell engagers, it is critical that they only cause lysis of the intended tumor cell populations. Candidates are screened for nonspecific binding to serum proteins using Surface Plasmon Resonance (SPR) and cell surface proteins by FACS

## Optimized ADAPTIR Scaffold Leads to Improved Mouse Pharmacokinetics

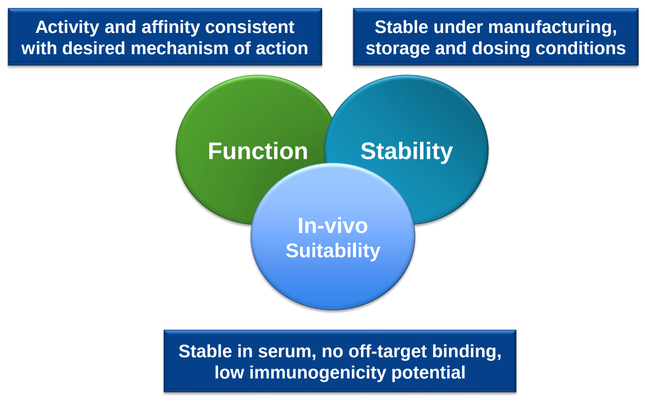



Stabilization and optimization of the ADAPTIR platform led to significant improvements in PK for a representative anti-tumor x anti-CD3 bispecific.

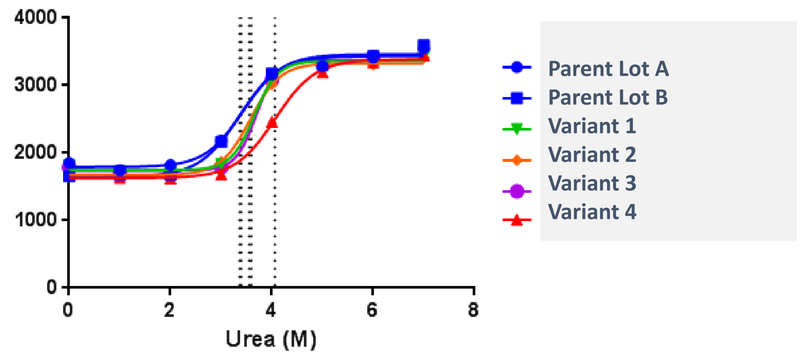
## Mass Spec is Utilized to Confirm Expected MW and Identify Unexpected PTMs




In the final stage of screening, constructs are tested by LC-MS and peptide mapping to confirm that the expected MW is achieved. In this example, proline hydroxylation occurred during stable, but not transient expression. This was addressed through sequence modification to replace the problematic residue.




## Evaluation of ADAPTIR Resistance to Urea Denaturation




The parent ADAPTIR and optimized variants were evaluated for their resistance to denaturation by increasing concentrations of urea. In addition to increased Tm (data not shown), the variants showed greater resistance to unfolding by urea, based on the shift in EC50 values derived from urea titrations up to 7 M.

## ADAPTIR Fc Mutations Eliminate Effector Function, but Retain Stability and FcRn Binding

### Thermal Stability

| Molecule | CH2 Tm (°C) | CH3 Tm (°C) | Functions |
|---|---|---|---|
| IgG1 Fc WT | 70 | 82 | ADCC/CDC capable |
| ADAPTIR | 71 | 83 | ADCC/CDC null |

### FcRn Affinity

| Molecule | IgG subtype | Molecule Type | KD by SPR (nM) |
|---|---|---|---|
| ADAPTIR | ADAPTIR IgG1 Fc (ADCC and CDC null) | ADAPTIR bispecific | 19 |
| Trastuzumab | IgG1 WT | MAb | 64 |

Mutations to eliminate binding to complement and Fc receptors were verified to maintain thermostability of the CH2 and CH3 domains and binding to FcRn.

## Optimization of Manufacturability of ALG.APV-527 (Anti-41BB x Anti-5T4)

| Construct | High Salt Solubility, % Protein Loss | Shear Stress, % Protein Loss | Tm (°C) 4-1BB scFv | Tm (°C) 5T4 scFv | In vitro Activity (EC50) |
|---|---|---|---|---|---|
| Parent | 89 | 64 | 56.1 | 70.5 | 0.23 |
| Variant A | 67 | 80 | 57 | 70.5 | 0.09 |
| Variant B | 55 | 32 | 60.4 | 71.0 | 0.03 |
| **Variant C** | **13** | **23** | **61.5** | **73.2** | **0.03** |

Following screening, optimization can be performed to address specific liabilities. Using accelerated stress conditions, variants were discovered that improve stability, while maintaining or improving biological activity. Increases in solubility, shear stability and thermostability were achieved via optimization of ALG.APV-527 (anti-41BB x anti-5T4 ADAPTIR bispecific).

## Summary

- ADAPTIR bispecific candidates are screened through a robust set of assays to identify leads that are active, stable and manufacturable
- Improvements in the ADAPTIR platform have resulted in candidates with improved PK and greater colloidal and conformational stability