Introduction
Treatment of metastatic, castrate-resistant prostate cancer (mCRPC) remains a highly unmet medical need. We developed a humanized bispecific molecule, built on the ADAPTIR™ modular protein technology platform, which redirects T-cell cytotoxicity against cells expressing PSMA (Prostate Specific Membrane Antigen), a prostate cancer antigen. PSMA was selected as the target due to its close association with prostate cancer, disease progression, and overall disease outcome.

Long Term In Vitro Cytotoxicity Assays
Reconstituted PBMCs in long-term cytotoxicity assays with ES414-B2B and ES414-C2B, in the presence or absence of target cells. PBMCs were incubated for 72 hours with target cells at a ratio of 3:1. ES414 (100 pM) was added to induce T-cell activation. T-cell proliferation was analyzed by loss of CFSE fluorescence. The graphs show the fraction of T cells proliferating in the presence or absence of target cells.

Assessing ES414 Binding to PBMC Subsets
ES414 binds to human PBMC subsets in a dose-dependent manner. The graphs show the fraction of T cells (CD8+ and CD4+), B cells (CD19+), and NK cells (CD56+) that are bound by ES414 at different concentrations.

Assessing ES414 Binding to FCyR Expressing Cells
Representative microscopy images show specific binding induced by ES414. U937 cells were incubated with ES414 and stained with Alexa Fluor 488-conjugated anti-human IgG to visualize specific binding. The images show the expression of FCyRs on U937 cells.

In Vitro T-Cell Proliferation
Representative plots show the proliferation of T cells in the presence of ES414 and target cells. The graphs show the fraction of T cells that proliferate in the presence of target cells and ES414 at different concentrations.

Summary and Conclusions
• ES414 selectively bound to T cells, and did not bind to other PBMC subsets or to FCyR expressing cell lines.
• T cells activated by ES414 in the presence of PSMA+ target cells rapidly induced target cell lysis within 24 hours and released interferon-γ. For more than 56 hours, target cell lysis occurred at low effector-to-target cell ratios.
• In the presence of target cells, ES414-induced T-cell activation, as measured by upregulation of activation markers (not shown), and proliferation, however, ES414 induced less cytokine release in comparison to control molecules and targets alternative bispecific antibody platform (siFv-TCR).

References
1. Miller, RE et al, Anti-PSMA x Anti-CD3 ADAPTIR™ molecule, ES414, in the absence of significant cytokine release.
7. Miller, RE et al, Anti-PSMA x Anti-CD3 ADAPTIR™ molecule, ES414, in the absence of significant cytokine release.
10. Miller, RE et al, Anti-PSMA x Anti-CD3 ADAPTIR™ molecule, ES414, in the absence of significant cytokine release.