# Bi-specific Antibodies Targeting Signaling Pathway Crosstalk are a New Breast Cancer Immunotherapeutic Strategy

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## Abstract

Most of the currently approved therapeutic anti-cancer antibodies are monospecific and therefore only capable of interfering with the biological function of a single molecular target. However, breast cancers mostly involve crosstalk of often synergistic signal transduction pathways, and thus, isolated blockade of a single signal transduction pathway is frequently met by escape mechanisms, such as upregulation of redundant pathways, rendering the monospecific immunotherapy less effective.

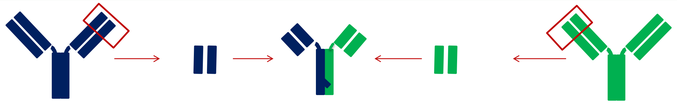
Using both chemical and molecular biology techniques, Sorrento has developed new approaches to generate IgG-like bi-specific antibodies (BsAbs) targeting either two compensating signal transduction pathways, such as HER family members, or a breast cancer specific antigen and an immuno-regulatory molecule such as PD-L1 or PD1. The chemical biology method, which involves specific hetero-dimerization of two half antibody molecules using bio-orthogonal chemistry, was used to generate an anti-c-Met and anti-PD-L1 chemical bi-specific antibody (CBA). Lastly, employing a molecular biology approach, an anti-c-Met and ErbB3 scFv-Fc bi-specific antibody was produced. Progresses on *in vitro* characterization and cell-based functional assays of these BsAbs are presented .

## Introduction

To overcome dose limiting toxicities and to increase efficacy of immunotherapy of cancer, various strategies have been development for selectively redirecting effector cells/molecules towards tumor cells. Many of these strategies exploit the specificity of tumor associated antigen recognition by monoclonal antibodies. Using either hybridoma fusion, chemical derivatization or molecular biology technology, antibodies with dual specificity can be constructed. These so called bispecific antibodies have been used to redirect the cytolytic activity of a variety of immune effector cells such as cytotoxic T lymphocytes, natural killer cells, neutrophils and monocytes/macrophages to tumor cells [1].

Several clinically available therapeutic monoclonal antibodies (mAbs) can induce immune-mediated tumor cell killing through mechanisms that include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). In clinical studies, ADCC has been demonstrated to significantly enhance the efficacy of various mAbs, including rituximab (anti-CD20), trastuzumab (anti-human-epidermal-growth-factor receptor 2 (Her2)) and cetuximab (anti-epidermal-growth-factor receptor (EGFR)) [2]. In order to enhance cell-mediated cytotoxicity, bispecific antibodies have recently been developed as new agents for immunotherapy [3, 4].

## Generation of scFv-Fc Knob-into-Hole BsAb

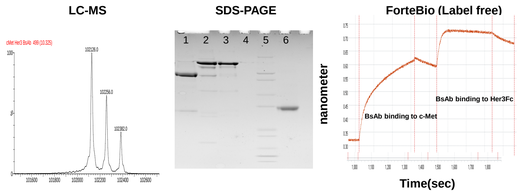


Anti-c-Met IgG1 converted to scFv | Anti-c-Met /anti-ErbB3 BsAb | Anti-ErbB3 IgG1 converted to scFv

**Affinity matured human anti-c-Met and anti-ErbB3 IgG1s are converted to scFv, and fused to Fc that carries mutations in constant domain 3 promoting heterodimerization.**

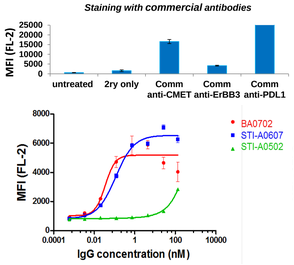
## Characterization of scFv-Fc Knob-into-Hole BsAb

### Analysis of the scFv-Fc BsAb BA-0702



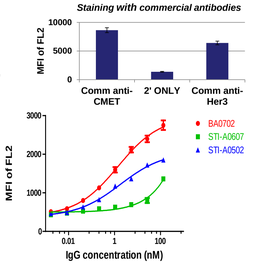

**Basic characterization of BA-0702** MS spec analysis confirmed the molecular weight of BA-0702 that is against c-Met and ErbB3; SDSPAGE (lane1 BA-0702, non-reducing and lane 6 BA-0702 reducing. Lanes 2 and 3: commercial IgG1 as control) confirmed expression and purity. Label free technology confirmed binding of both antigens by BA-0702 at the same time.

### BA-0702 binding to MDA-MA-468




| | BA-0702 | STI-A0607 | STI-A0502 |
|---|---|---|---|
| $EC_{50}$ (nM) | 0.035 | 0.11 | - |

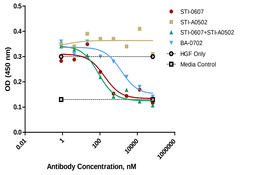
### BA-0702 binding to MCF7




| | BA-0702 | STI-A0607 | STI-A0502 |
|---|---|---|---|
| $EC_{50}$ (nM) | 1.4 | >100 | 1.8 |

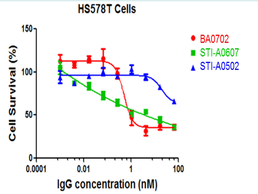
**Enhanced BA-0702 binding to cancer cells expressing c-Met and/or ErbB3**. top panels show by FACS the expression of c-Met and ErbB3 in TNBC (MDA-MA-468) and MCF7 cells. Lower panels show by FACS dose dependent binding of BA0702 and parental IgG1s to the cells.

### c-Met Phosphorylation Inhibition by BA-0702




| | STI-A0607 | STI-A0502 | STI-0505+ STI-A0502 | BA-0702 |
|---|---|---|---|---|
| $IC_{50}$ (nM) | 175.3 | NA | 82.9 | 1360 |

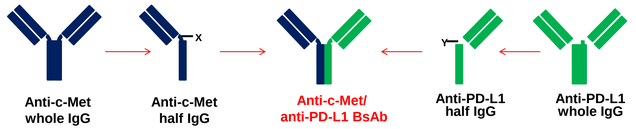
### Potential Application of BA-0702 for ADC




| | BA-0702 | STI-A0607 | STI-A0502 |
|---|---|---|---|
| $IC_{50}$ (nM) | 0.50 | NA | >60 |

**BA-0702 mediated inhibition of c-Met signal transduction pathway and enhanced cell killing when complexed with toxins**. Top panel shows BA-0702 with one anti c-Met arm can suppress HGF induced c-Met phosphorylation. Bottom panel shows the testing of the antibodies in a cell-based cytotoxic assay after complex formation with Protein G-MMAF. The improved killing observed with BA-0702 illustrates the potential of the BsAb to be used in an ADC format.

## Generation of BsAb by Chemical Conjugation

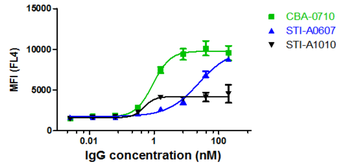


Anti-c-Met whole IgG | Anti-c-Met half IgG | Anti-c-Met/ anti-PD-L1 BsAb | Anti-PD-L1 half IgG | Anti-PD-L1 whole IgG

**An anti-PD-L1 and anti-c-Met bispecific antibody was generated via a conjugation between two chemically modified half antibody molecules.**

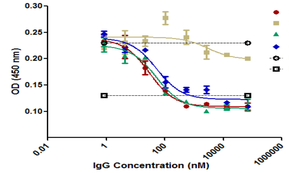
## Characterization of Chemical Conjugation BsAb

### Anti-c-Met/PD-L1 BsAb CBA-0710 Induces Enhanced Binding



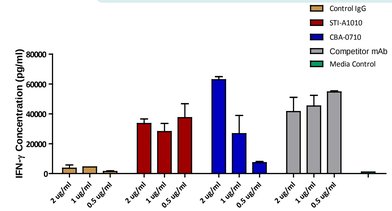

**Chemical BsAb CBA-0710 binding on MDA-MB-231 cells.** The cells express both c-Met and PD-L1. STI-A0607 is an anti-c-Met mAb, STI-A1010 is an anti-PD-L1 mAb.

### Inhibition of c-Met Phosphorylation by Anti-c-Met/PD-L1 BsAb CBA-0710

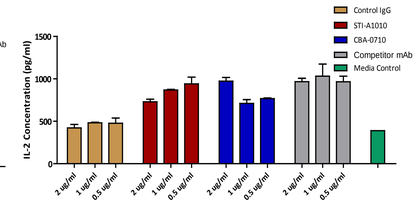



**Phosphorylation in MDA-MB-231 cells.** CBA-0710 is an anti-c-Met/PD-L1 BsAb, STI-0607 is an anti-c-Met mAb, STI-A1010 is an anti-PD-L1 mAb.

### *In vitro* Immunomodulation by Anti-c-Met/PD-L1 BsAb CBA-0710




**Increased IFN-γ release in response to anti-c-Met/PD-L1 BsAb**




**Increased IL-2 release in response to anti-c-Met/PD-L1 BsAb**

## Conclusions

- **Bi-specific antibodies of many different formats have been developed with superior anti cancer activities, and one of the major obstacles in producing BsAb is the so-called chain association issue [5]. We have shown here that scFv fragment based approach and conjugation based method are viable methods of generating BsAbs.**
- **Anti-c-Met/ErbB3 BsAb BA-0702 in scFv-Fc format demonstrated superior binding activity towards tumor cell lines MDA-MA-468 and MCF7 to each parental monospecific IgG1s. Also, BA-0702 showed activity in suppressing HGF induced c-Met phosphorylation in cancer cell line MDA-MB-231; and showed much higher cell killing activity (HS578T) than each parental IgG1s when they all were complexed with MMAF-conjugated Protein-G, indicating its potential application in ADC.**
- **Similarly, Anti-c-Met/PD-L1 chemical BsAb CBA-0710 retained excellent affinity for their respective cellular target and demonstrated potent *in vitro* activities in cell-based functional assays.**

## References

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2. Lameris et al. Bispecific antibody platforms for cancer immunotherapy. *Critical Reviews in Oncology/Hematology. 2014. In press.*
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