

Abstract

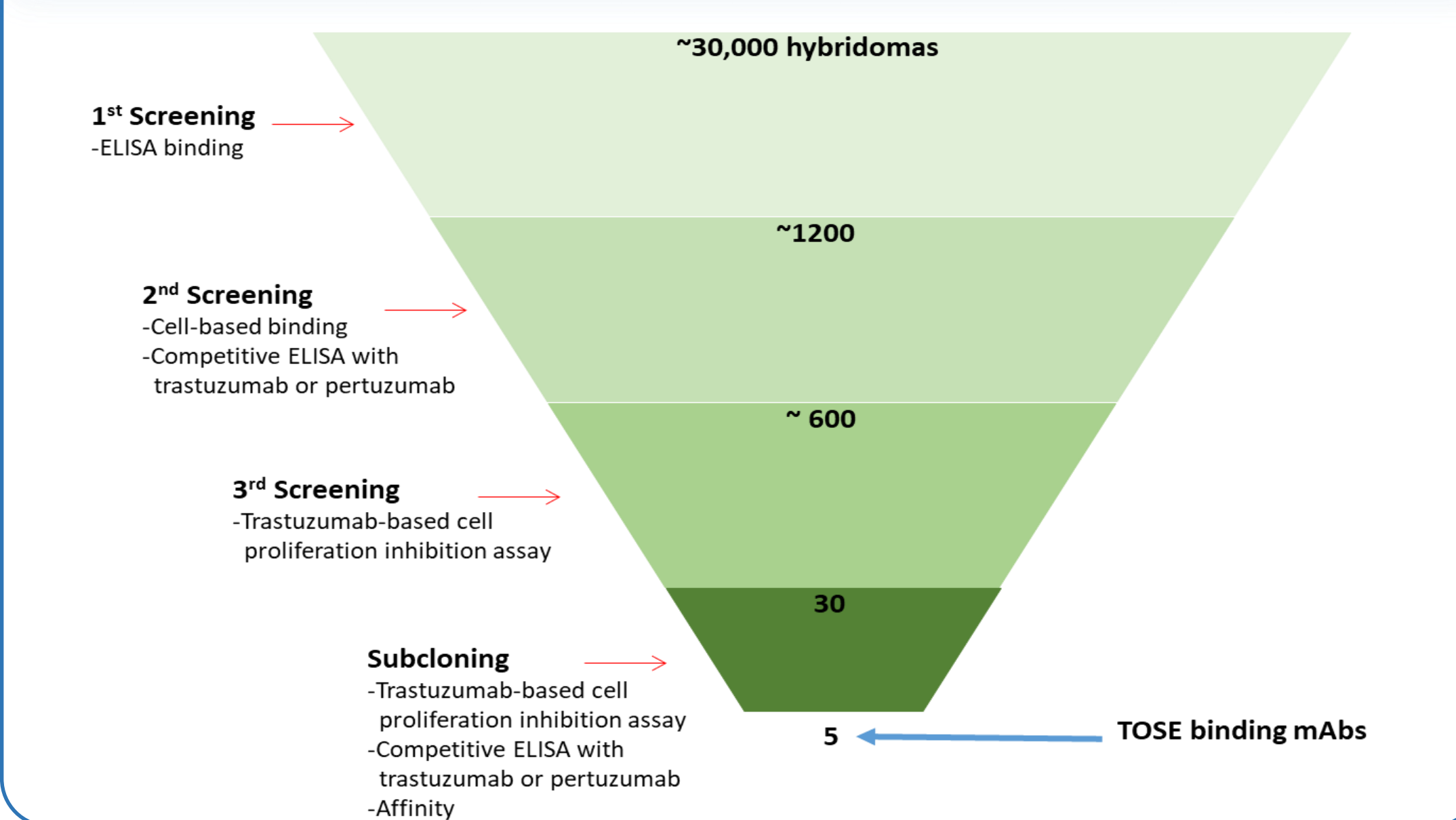
Background: Trastuzumab and pertuzumab are anti-HER2 antibodies that bind to two different epitopes of HER2. Although pertuzumab itself did not exhibit substantial clinical benefit as monotherapy, it was approved to be used in combination with trastuzumab because of its ability to enhance the clinical efficacy of trastuzumab. Currently, the combination of trastuzumab and pertuzumab is being used as first-line treatment for HER2-positive metastatic breast cancer. However, while the combination of trastuzumab and pertuzumab has been effective, there is still opportunity for greater effectiveness by identifying anti-HER2 mAbs that will be better synergistic partners of trastuzumab, allowing for better efficacy and/or broader indications.

Methods: A SynAb™ Platform was used to identify BSI-001, an anti-HER2 mAb, through trastuzumab-based synergistic functional screening. A competitive ELISA was used to perform epitope binning of the anti-HER2 antibodies. BSI-001 was further characterized in combination with trastuzumab for its bioactivity *in vitro*, including inhibition of cell proliferation and antigen-mediated internalization of HER2 positive cell lines. The synergistic effect of BSI-001 with trastuzumab was also evaluated in trastuzumab-resistant cell lines. Multiple animal models were then employed to investigate the synergistic anti-tumor activity of the combination *in vivo*.

Results: BSI-001 is the best functional partner of trastuzumab identified through the SynAb™ Platform. BSI-001 binds to a unique epitope, distinct from the binding epitopes of trastuzumab and pertuzumab. It exhibits superior bioactivity and efficacy *in vitro* and *in vivo* when combined with trastuzumab. The combination of BSI-001 and trastuzumab inhibits HER2-positive cancer cell growth more potently than that of trastuzumab and pertuzumab. The combination of BSI-001 with trastuzumab also shows inhibition of cell growth of the trastuzumab-resistant cell line BT-474 Clone 5, whereas the combination of pertuzumab and trastuzumab does not. In addition, BSI-001 is able to synergize with trastuzumab to promote antigen-antibody internalization. In animal models, BSI-001 demonstrates superior synergistic anti-tumor activity when combined with trastuzumab as compared to pertuzumab.

Summary: The combination of BSI-001 and trastuzumab showed significantly better *in vitro* and *in vivo* activity than the combination of trastuzumab and pertuzumab. In addition, because of the activity of the BSI-001/trastuzumab combination in a trastuzumab-resistant cancer cell line, there is the potential for this combination to be used to treat patients with trastuzumab-resistant cancer or patients who experienced relapse following trastuzumab and pertuzumab treatment.

Screening of Trastuzumab-Optimal-Synergistic-Epitope (TOSE)-Binding Monoclonal Antibodies

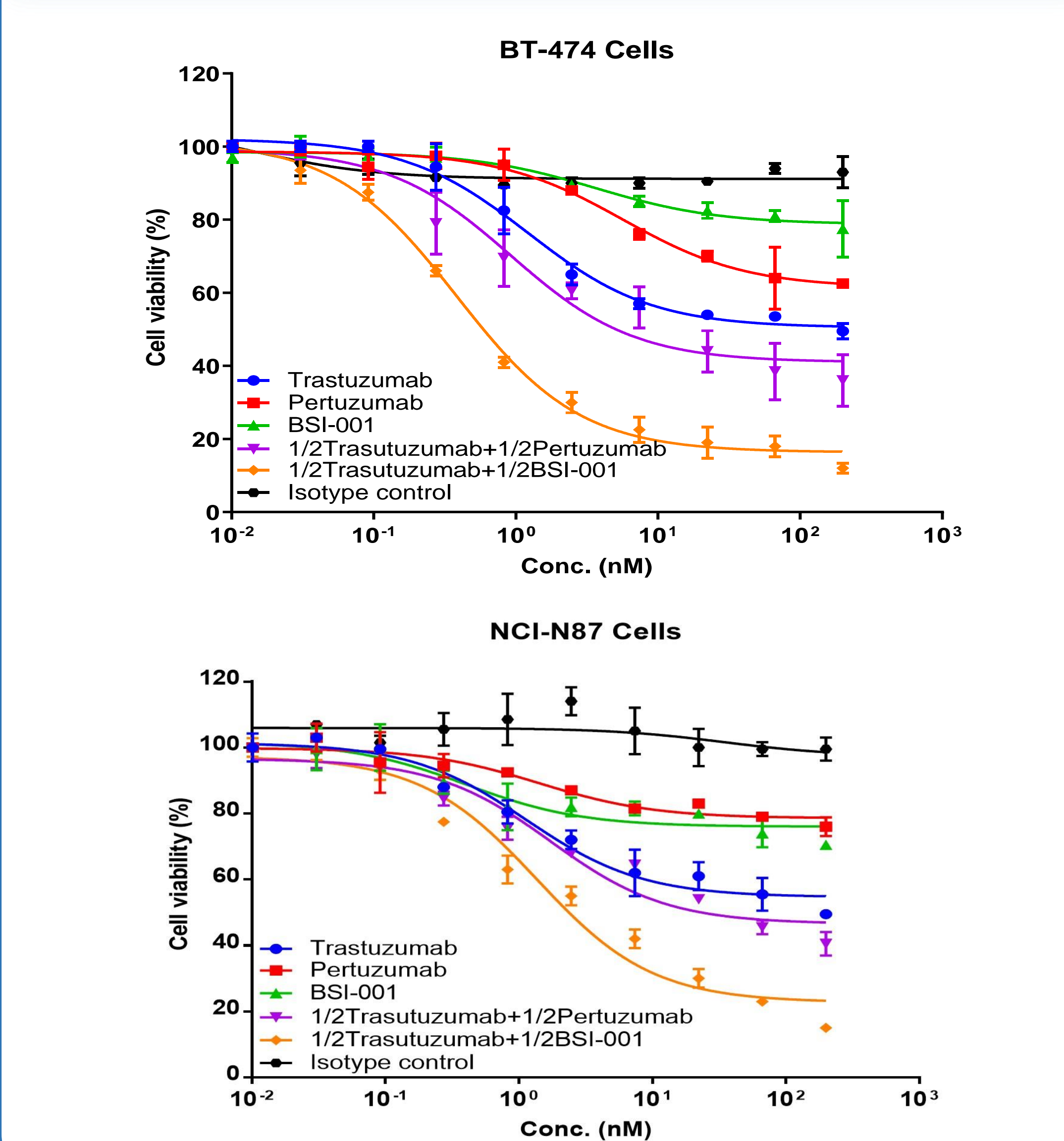


Antibodies Bind to a Unique Epitope Distinct from That of Trastuzumab or Pertuzumab

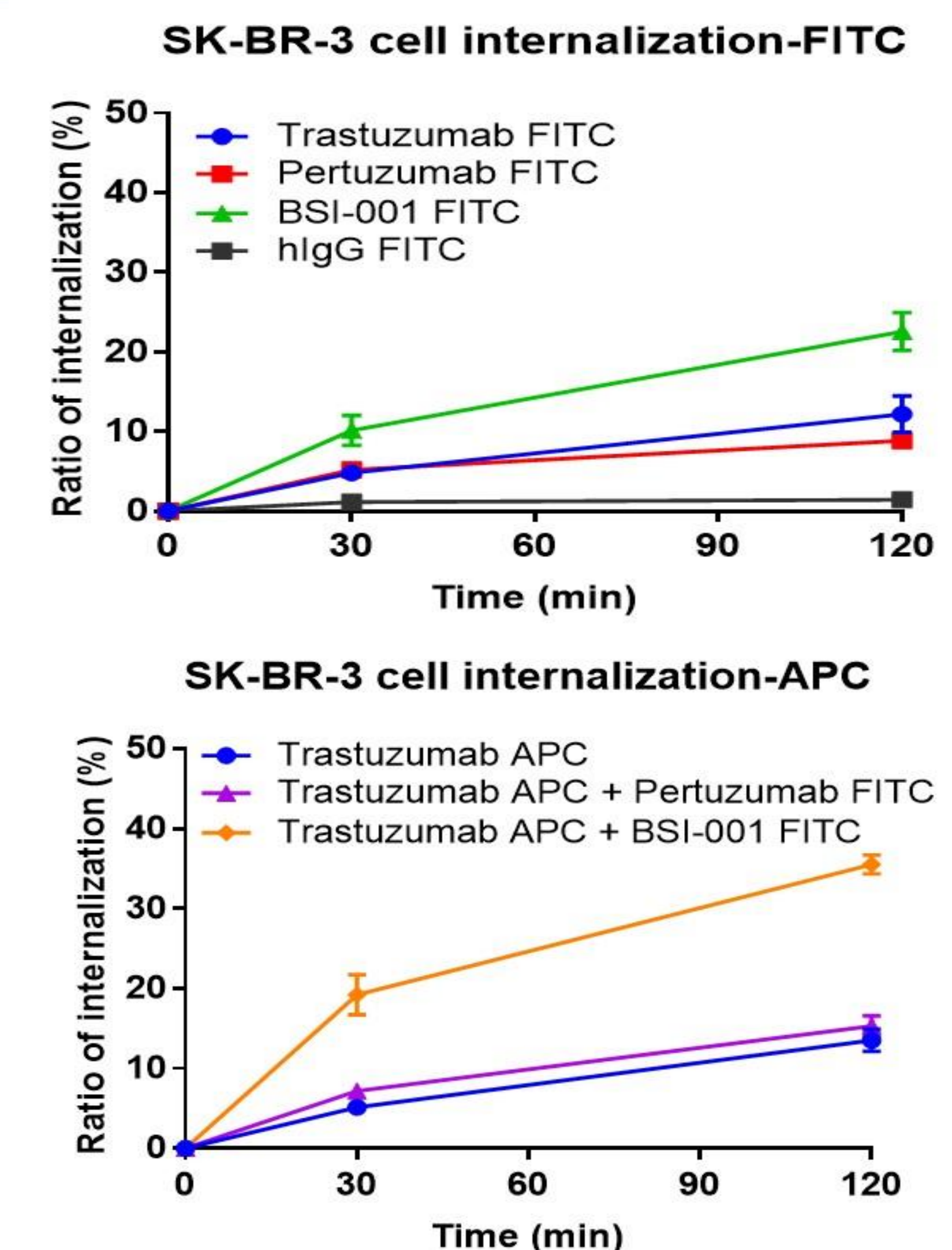
	4C9	4H2	4G6	5F12	BSI-001	Trastuzumab	Pertuzumab
4C9	0.05	0.12	0.05	0.15	0.12	0.52	0.51
4H2	0.08	0.03	0.07	0.1	0.09	0.57	0.49
4G6	0.04	0.09	0.04	0.11	0.11	0.47	0.51
5F12	0.05	0.07	0.06	0.02	0.09	0.41	0.41
BSI-001	0.03	0.05	0.05	0.04	0.03	0.42	0.39
Trastuzumab	0.87	0.63	0.49	0.56	0.49	0.03	0.33
Pertuzumab	0.75	0.81	0.75	0.75	0.62	0.87	0.64

Legend: Binding (white), Blocking (red), Self-blocking (green)

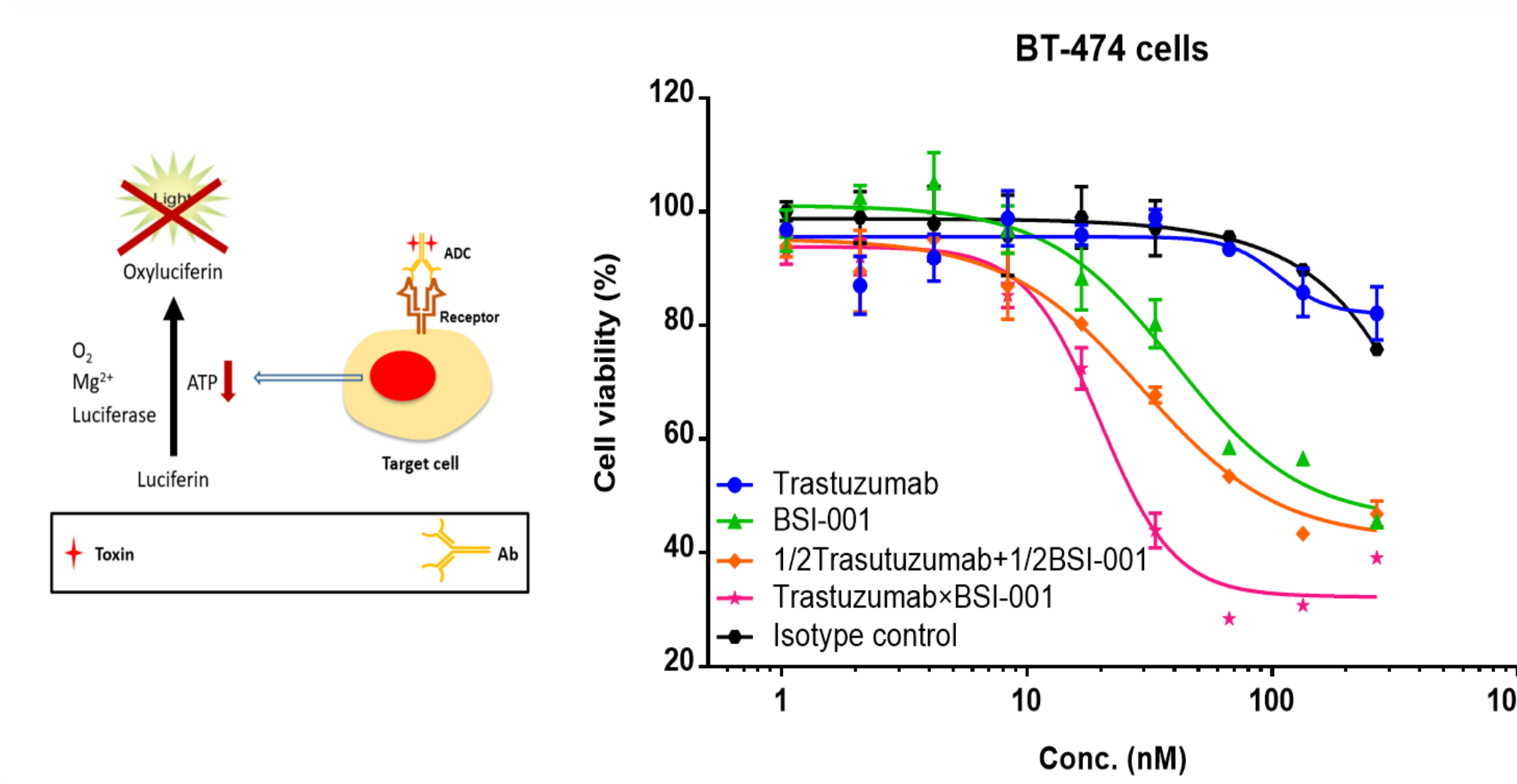
BSI-001 and Trastuzumab Synergistically Inhibit HER2-Positive Cancer Cell Growth



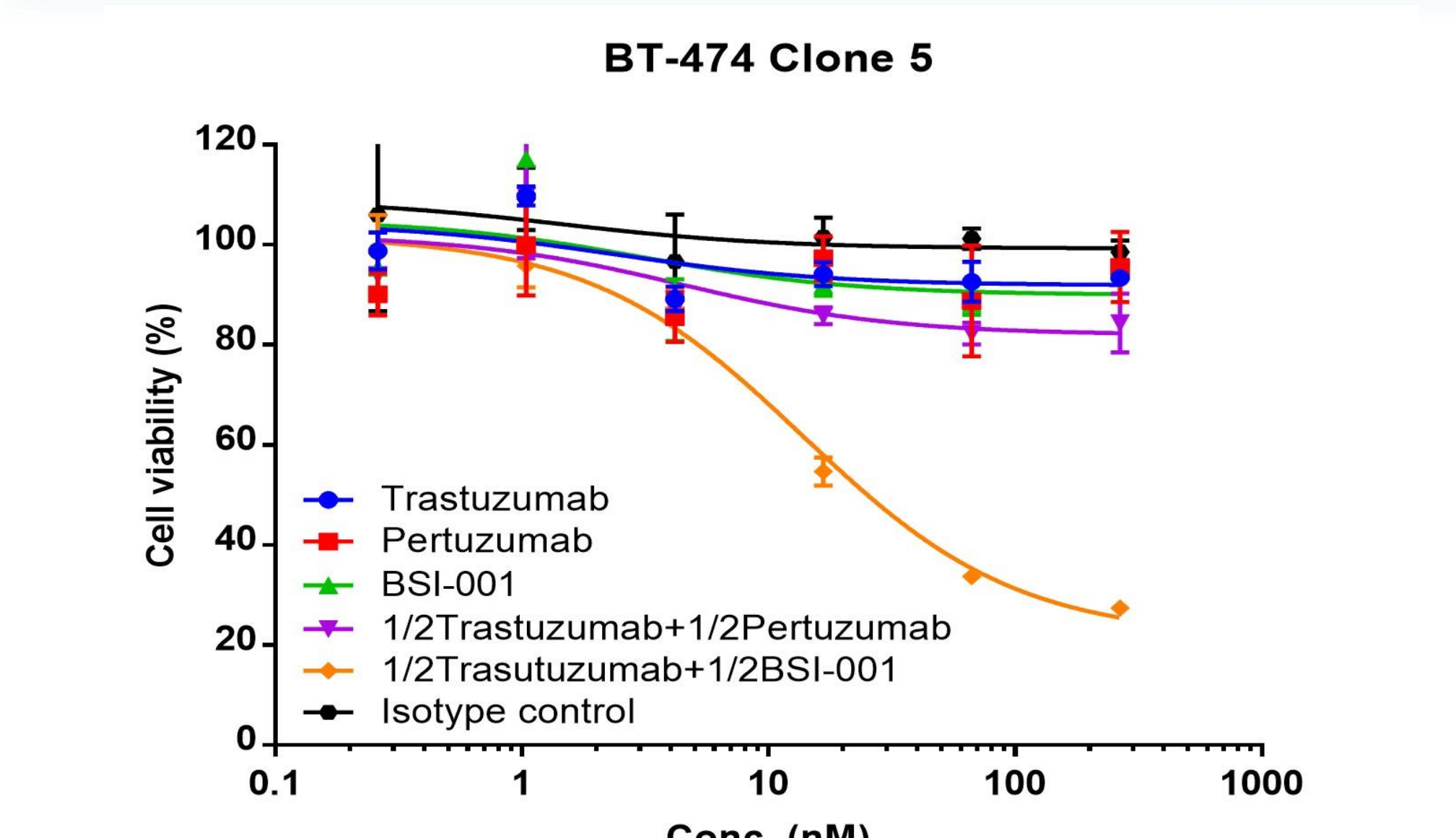
BSI-001 and Trastuzumab Enhance HER2 Internalization



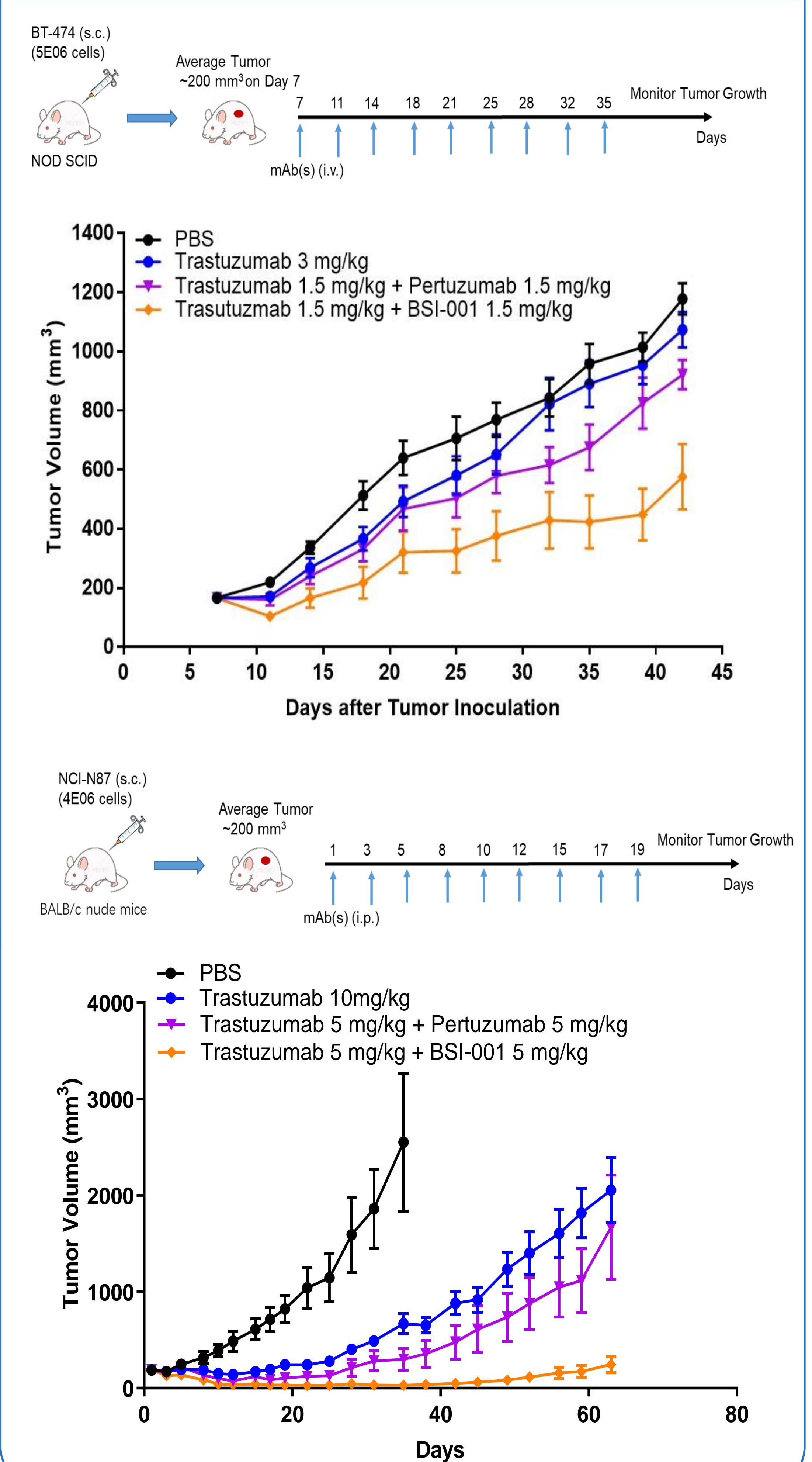
BSI-001/Trastuzumab Biparatopic Antibody Exhibits High Internalization Activity



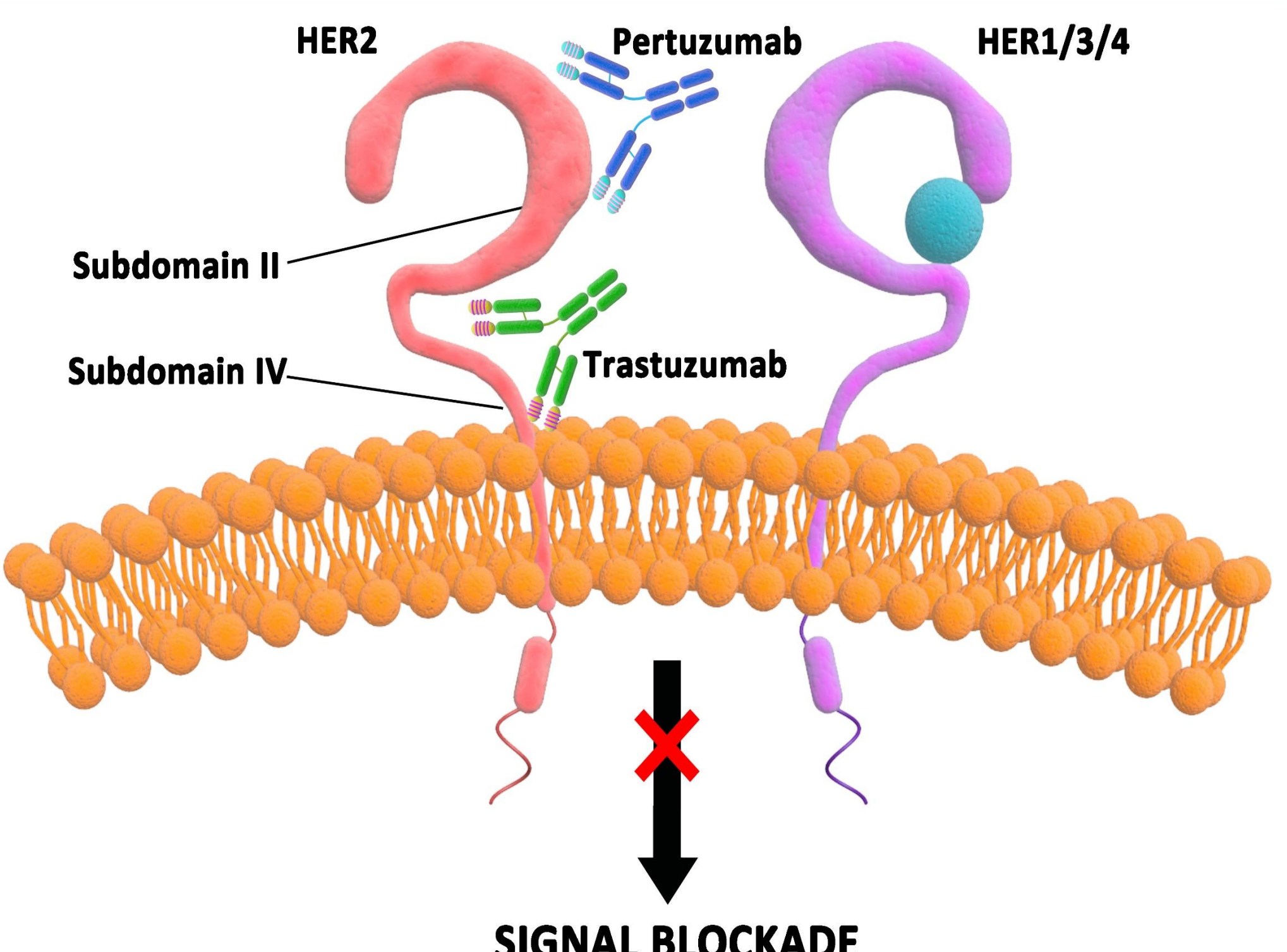
BSI-001 and Trastuzumab Synergistically Inhibit Trastuzumab-Resistant Cell Growth



BSI-001 and Trastuzumab Exhibit Superior *in vivo* Anti-Tumor Synergistic Efficacy



A Dual Blockade of HER2-Driven Signaling Pathways by Trastuzumab and Pertuzumab



Conclusions

- BSI-001, a best-in-class synergistic partner of trastuzumab, demonstrates significant higher *in vitro* and *in vivo* bioactivity than pertuzumab when combined with trastuzumab;
- Unlike pertuzumab, BSI-001 combined with trastuzumab shows significant growth inhibition of trastuzumab-resistant cells, highlighting a potential therapy to trastuzumab-resistant patients;
- BSI-001 or BSI-001/trastuzumab biparatopic antibody exhibits significantly higher internalization activity than trastuzumab, indicating their potential use for next generation of HER2 ADCs.