BGB149, a novel clinical stage humanised anti-Axl function blocking antibody

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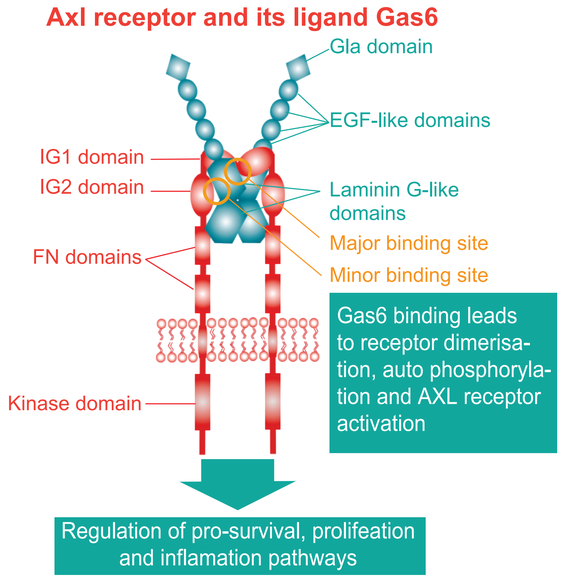
In collaboration with:

Background

- Axl is a member of the TAM (TYRO3-AXL-MER) receptor tyrosine kinase (RTK) family.
- Growth arrest-specific 6 (Gas6) is the main Axl ligand.
- The TAM RTKs regulate a wide range of cellular responses such as inflammation, proliferation and cell survival.
- Axl is overexpressed in several hematological and solid malignancies, including acute myeloid leukemia, triple negative breast cancer, non-small cell lung cancer and prostate cancer.
- Patients with high levels of Axl expression often display more aggressive and metastatic disease and have worse overall survival.
- Axl is known to be important in epithelial to mesenchymal plasticity, metastasis, tumor immune evasion and resistance to chemotherapeutic and targeted drugs.
- Axl is also an important regulator of innate immune responses, serves to dampen physiological inflammatory processes and is often expressed on pro-tumorigenic tumor infiltrating macrophages.

Axl receptor and its ligand Gas6



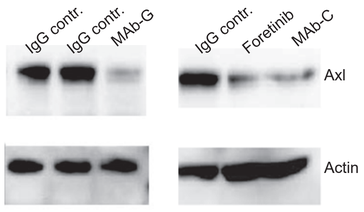
BGB149 - antibody development program

Brief overview:

- >30 mouse hybridomas generated using two approaches:
  - DNA immunization
  - AXL protein immunization
- Three Axl function-blocking antibodies selected: MAb-C, MAb-G and MAb-F. MAb-H was selected as diagnostic candidate.
- Highly selective to human Axl, no cross-reactivity with Mer and Tyro3.
- All Mabs bind human Axl with sub-nM affinity (5-500pM).
- The 4 selected antibodies were cloned and sequenced, and this sequence information was used to generate chimeric antibodies (cMAb's).
- Four fully humanized variants of antibody MAb-G were generated (MAb-G H1L1, MAb-G H1L2, MAb-G H2L1, MAb-G H2L2).
- The humanized antibodies demonstrated similar binding characteristics to the parent murine antibody MAb-G.
- Candidate therapeutic BGB149 developed from H2L1.

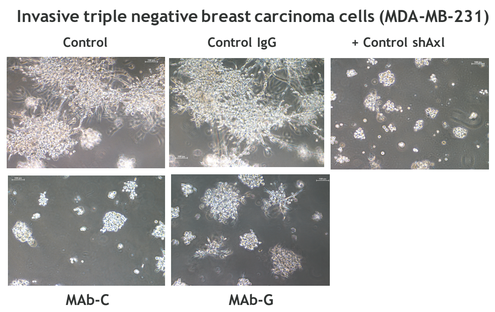
Discovery Phase

Downregulation of AXL by anti-AXL MAbs



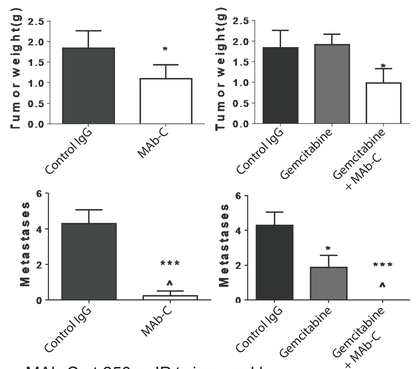
MAb-C and MAb-G down-regulate Axl in MDA-MB-231 cells

MAbs prevent invasive cell growth



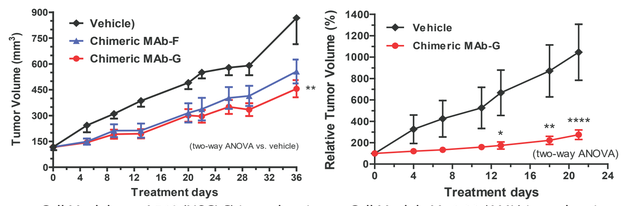
AXL-MAbs demonstrate anti-tumor activity in 3D cell cultures

MAbs reduce pancreatic Mia PaCa-2 tumor growth and block metastasis



MAb-C at 250ug IP twice weekly
Gemcitabine at 25mg/kg IP twice weekly
Kirane *et al.*, Cancer Res. 2015

Chimeric Axl-MAbs are effective as single agents in mouse xenograft models

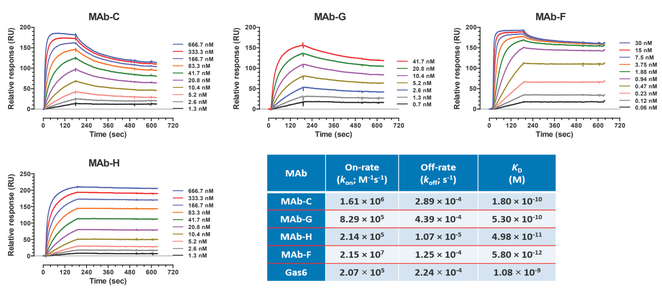


Cell Model : s.c. A549 (NSCLC) in nude mice
MAbs: 20 mg/kg, i.p., q4d, 4 wks

Cell Model : Mv4-11 (AML) in nude mice
MAb: 30 mg/kg, i.p., q4d, 4 wks

Axl-MAbs show efficacy as single agents in mouse models of human NSCLC and AML.

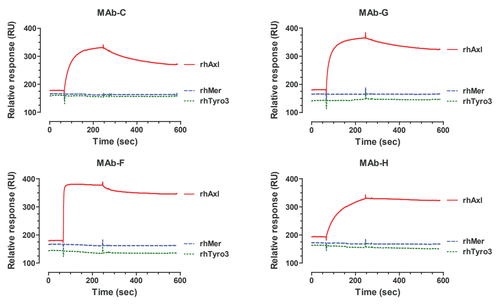
All MAbs bind human Axl with sub-nM affinity



MAb	On-rate (k_{on}; $M^{-1}s^{-1}$)	Off-rate (k_{off}; s^{-1})	K_D (M)
MAb-C	1.61×10^6	2.89×10^{-4}	1.80×10^{-10}
MAb-G	8.29×10^5	4.39×10^{-4}	5.30×10^{-10}
MAb-H	2.14×10^5	1.07×10^{-5}	4.98×10^{-11}
MAb-F	2.15×10^7	1.25×10^{-4}	5.80×10^{-12}
Gas6	2.07×10^5	2.24×10^{-4}	1.08×10^{-9}

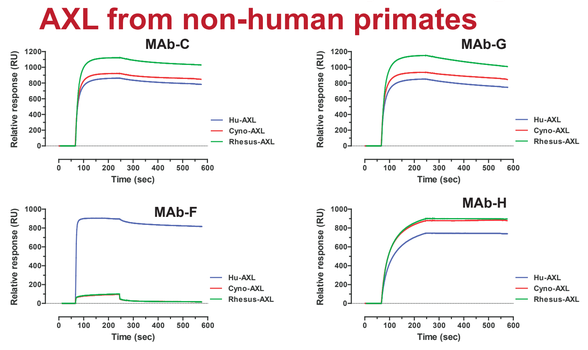
Kinetic analysis of selected MAbs.

All MAbs specifically bind human AXL, no binding to Mer and Tyro3



Binding analyses showing interactions of MAbs to recombinant human TAM receptors (Axl, Mer, Tyro3) immobilized on the surface of a censor chip.

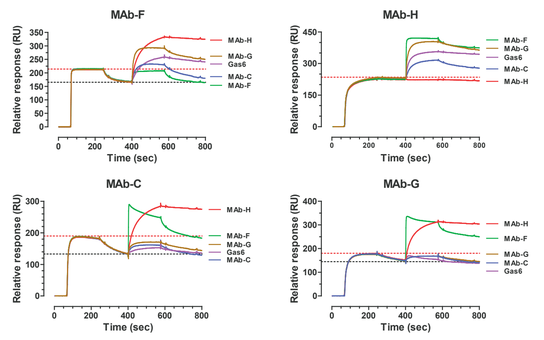
Species cross-reactivity: binding to AXL from non-human primates



AXL-MAbs C, G and H specifically bind AXL from cynomolgus and rhesus monkeys.

None of the MAbs bind to mouse Axl (results not shown)

Cross-competition - Epitope mapping



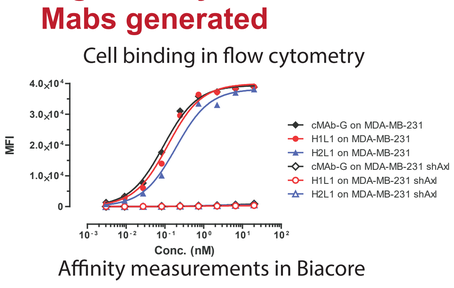
Group 1: MAb-F
Group 2: MAb-H
Group 3: MAb-C and MAb-G. Inhibit Gas6 binding

First sample \ Second sample	MAb-F	MAb-H	MAb-C	MAb-G	Gas6
MAb-F	+	-	-	-	-
MAb-H	-	+	-	-	-
MAb-C	-	-	+	+	+
MAb-G	-	-	+	+	+

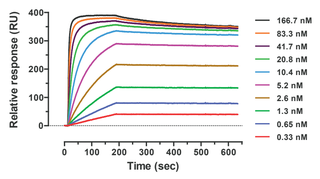
Characterization of humanized antibody

High affinity humanized Axl Mabs generated

Cell binding in flow cytometry



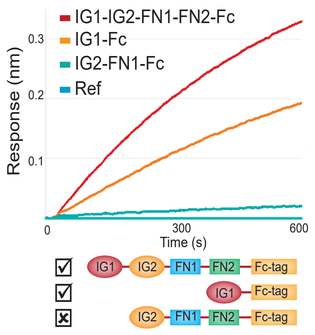
Affinity measurements in Biacore



MAb	On-rate (k_{on}; $M^{-1}s^{-1}$)	Off-rate (k_{off}; s^{-1})	K_D (M)
H1L1	1.54×10^6	1.61×10^{-4}	1.05×10^{-10}
H2L1	2.07×10^6	1.42×10^{-4}	6.86×10^{-11}
cMAb-G	1.07×10^6	1.61×10^{-4}	1.50×10^{-10}

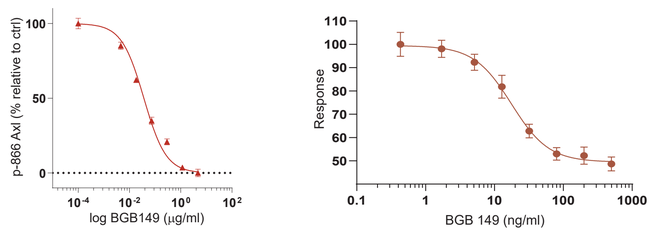
BGB149 binds to the IG1 domain in AXL

Epitope mapping – truncation analysis



Binding of AXL truncations to BGB149 on Octet. BGB149 binds to IG1, the domain which has the major Gas6 binding site.

BGB149 dose dependently block Gas6 mediated AXL phosphorylation and block binding of Gas6 to Axl

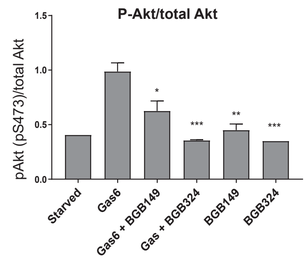


Starved HeLa cells were treated with BGB149 at indicated dosages for 1h prior to stimulation with crosslinked Gas6 for 20 min.

Biological models	Relative IC_{50}
HeLa	0.03 µg/ml
MDA-MB-231	0.03 µg/ml

Binding of horseradish peroxidase labelled Gas6 to immobilised Axl in the presence of different concentrations of BGB149.

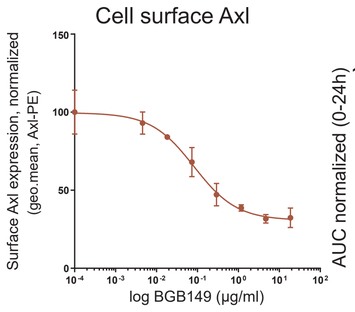
BGB149 prevents Gas6 mediated Axl-downstream signaling



Starved HeLa cells were treated with BGB149 (50 µg/ml) or bemcentinib (BGB324; 0.2 µM) for 1 hr prior to stimulation with crosslinked Gas6 (1 µg/ml) for 20 min. Cell lysates were analyzed by AKT (Total/Phospho) ELISA.

BGB149 dose dependently decreases surface Axl, leads to receptor internalization and reduces total Axl in tumor xenografts

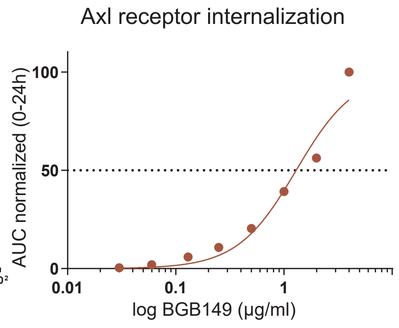
Cell surface Axl



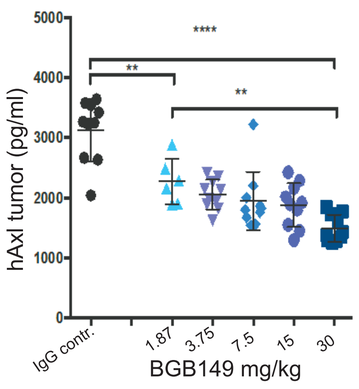
MDA-MB-231 cells were treated with BGB149 at indicated dosages for 20 hrs, and expression of surface AXL was evaluated by flow cytometry.

Biological models	Relative EC_{50}
HeLa	0.04 µg/ml
MDA-MB-231	0.01 µg/ml

Axl receptor internalization



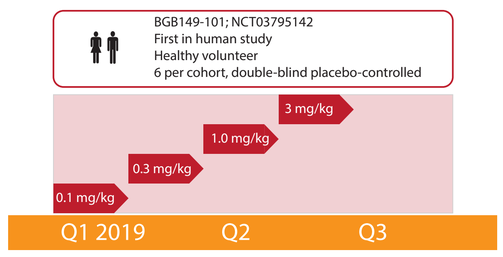
MDA-MB-231 cells were treated with BGB149 labeled with IncuCyte® FabFluor at indicated concentrations for 24 hrs. Red fluorescence images were captured every 30 min over 24 hrs.



Levels of AXL total protein in A549 xenograft tumors in SCID mice after treatment with IgG control at 30 mg/kg or BGB149 at indicated dosages twice weekly for 3 weeks.

Phase 1 clinical study

BGB149 is currently being evaluated in a Phase I clinical study



Phase I healthy volunteer trial BGB149-101 to investigate the safety, tolerability and pharmacokinetics of BGB149 following single dose administration.

BGB149 showed low toxicity in monkeys.
Phase 1 dose based on NOAEL 10 mg/kg/week in monkeys.

Summary

- A platform of AXL function-blocking antibodies: Lead and back-ups.
- Highly selective to human and non-human primate AXL. No cross-reactivity with other TAM members: Mer and Tyro3.
- High Affinity (K_D): 5 -500 pM (by Biacore)
- Blocks binding of Gas6 to Axl
- In vivo Anti-tumor efficacy demonstrated in animal models of disease: AML, NSCLC, pancreatic cancer.
- Low toxicity in monkeys.
- BGB149 is currently being evaluated in a Phase I clinical study.

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