Anti-tumor activity of the antibody-drug conjugate (ADC), BT062, against CD138-positive solid tumors

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Abstract

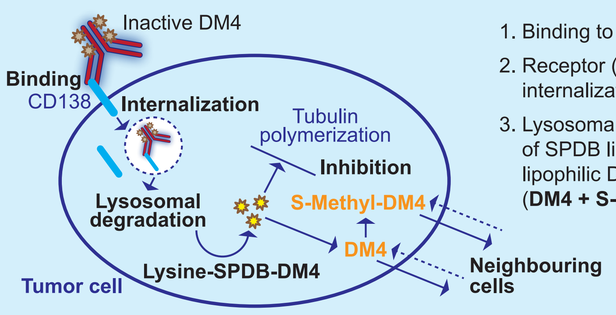
BT062 is an antibody drug conjugate (ADC) comprising a chimeric anti-CD138 antibody conjugated to the maytansinoid DM4. CD138 has long been recognized as being highly expressed on multiple myeloma (MM), and findings previously reported include the highly selective cytotoxic activity of BT062 against CD138-positive MM cells (Ikeda, *et al*. 2009) and safety and efficacy in MM in early clinical testing (Khan, *et al*. ASH 2009, Kelly, *et al*. ASH 2013).

Here, we show the potential of BT062 as a treatment for CD138-positive solid tumors. CD138 has been found to be highly expressed on a variety of tumor types with limited treatment options today, including triple-negative breast cancer and bladder cancer. BT062 demonstrates potent and selective activity against such cell lines *in vitro*. It has also been found to be highly active against CD138-positive solid tumors in xenograft *in vivo* models, including primary human mammary and transitional cell bladder carcinoma tumors.

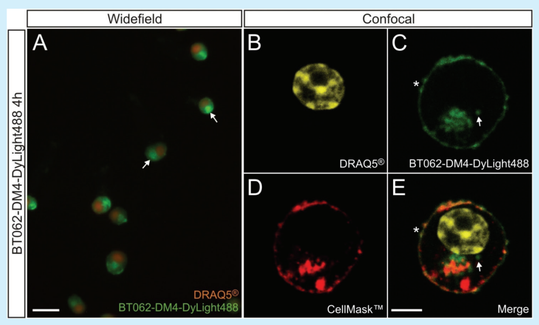
BT062 (indatuximab ravtansine)

- BT062 is an ADC based on the TAP (**T**argeted **A**ntibody **P**ayload) technology of ImmunoGen Inc.
- A hindered disulfide linker conjugates 1 anti-CD138 antibody to an average 3.5 molecules of the maytansinoid DM4, an inhibitor of tubulin polymerization.
- The conjugate is inactive in blood plasma; CD138-mediated processing releases DM4 which leads to inhibition of tubulin polymerization and cell death (Figure 1).

Figure 1. Mechanism of action of BT062



1. Binding to CD138
2. Receptor (CD138) mediated internalization
3. Lysosomal processing of SPDB linker leads to lipophilic DM4 metabolites (**DM4 + S-methyl-DM4**)
4. Inhibition of tubulin polymerization
5. Cell cycle arrest and apoptosis
6. Bystander killing



Internalization into CD138⁺ U-266 myeloma cells after 4 hours. BT062 was detected in a restricted manner in U-266 cells. Ongoing analyses with intracellular markers: EEA1 and LAMP-1.

A. PFA fixed U-266 cells after 4 hours of antibody incubation and staining of cell nuclei. Scale bar: 20 µm.

B – E. Confocal recording of a single U-266 cell 4 hours after antibody incubation and staining Arrows indicate defined aggregates of BT062-DM4-DyLight488 in the intracellular space. Asterisks mark membrane associated antibody. Scale bar: 5 µm.

CD138 (syndecan-1) in multiple myeloma and clinical trials of BT062

- CD138 (syndecan-1) is a transmembrane heparan sulfate proteoglycan which has a range of functions including mediation of cell proliferation, cell migration, and cell matrix interactions. It also contributes to tumor cell interaction with surrounding tissues.
- CD138 is highly upregulated on multiple myeloma cells and is used as a marker of disease and prognosis. Therefore, it represents a promising target for therapeutic intervention.
- A first in human study of BT062, administered intravenously (IV) once every 3 weeks to patients with relapsed or relapsed/refractory MM (Study 969), demonstrated an acceptable toxicity profile and evidence of clinical activity (Table 1).
- In the phase I/IIa (Study 975), 35 patients with relapsed or relapsed/refractory MM were treated on at a more frequent dose schedule (Table 1). BT062 continued to be well tolerated.
- Based on preclinical results, a phase I/IIa combination trial (Study 983) is ongoing to investigate the safety and efficacy of BT062 when administered in combination with lenalidomide and dexamethasone (Table 1).

Table 1. Clinical trials BT062 in multiple myeloma

Study No.	Indication/ Phase	Country	Design	Subjects planned/ enrolled	Status
969	Multiple myeloma: Phase I	USA	Monotherapy: First in Human, Repeated single dose, once every 3 weeks	32/32	Study is complete
975	Multiple myeloma: Phase I/IIa	USA	Monotherapy: Repeated multi dose, Days 1, 8, 15 every 4 weeks	35/35	Recruitment completed; approaching study closeout
983	Multiple myeloma: Phase I/IIa	USA	Combination therapy: Repeated multi dose, Days 1, 8, 15 every 4 weeks in combination with Len/dex	50/10	Recruitment ongoing

CD138 (syndecan-1) in solid tumor indications

- Besides multiple myeloma, syndecan-1 expression has been detected in several solid tumor types including pancreas, breast, prostate, colon, lung, endometrial, ovarian, head and neck, and bladder.
- In accordance with published data, immunohistochemistry studies of tumor samples from tissue arrays showed high CD138 expression in a variety of solid tumors (Table 2).

Table 2. Expression of CD138 in solid tumor samples

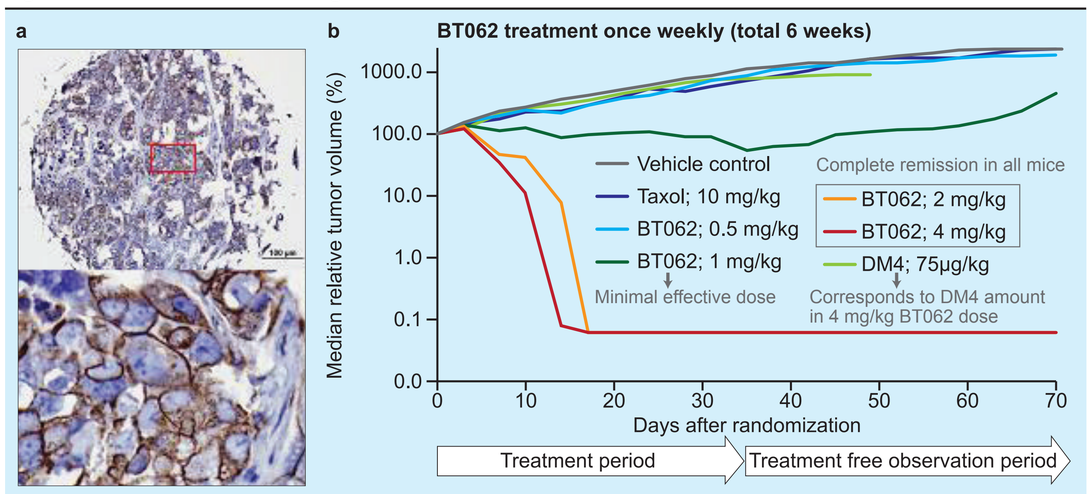
Tumor Type	Predominant Subtype		CD138 positive tumor samples*	
Bladder	Transitional Cell Carcinoma		63%	Analyzed in primary human tumor xenografts
Breast	Ductal Carcinoma: Invasive and in situ		45%	
Pancreatic	Adenocarcinoma		50%	
Lung	Non-small cell lung carcinoma	Large cell (LC) squamous subtype	55%	
		Adenocarcinoma	50%	
Prostate	Adenocarcinoma		50%	
Head and Neck	Squamous cell type		39%	Potential secondary indications
Cervix*	Squamous cell carcinoma		83%	
Hepatocellular	Hepatocellular carcinoma		99%	
Ovarian*	Adenocarcinoma		41%	
Colon*	Adenocarcinoma		61%	
Gastric	Adenocarcinoma		61%	
Thymus	Squamous cell carcinoma		75%	

- BT062 is highly potent and specific against CD138-positive multiple myeloma cells, therefore preclinical studies were conducted to investigate the potential of BT062 for the treatment of CD138 expressing solid tumors.
- Tumors from selected indications were further characterized in patient derived xenograft models. Therefore, primary human tumor fragments were transplanted onto nude mice. When a tumor volume of approximately 100 mm^3 was reached, treatment was started.

Anti-tumor activity of BT062 against CD138 expressing mammary carcinoma in a mouse xenograft model

- IHC analysis confirmed CD138 expression on a mammary carcinoma derived from a Her2/neu negative patient, refractory to hormone therapy. (Figure 2a).
- NMRI nude mice were subcutaneously implanted with the CD138-positive mammary tumor.
- BT062 (0.5 mg/kg, 1 mg/kg, 2 mg/kg, or 4 mg/kg) administered intravenously once weekly (total 6 injections).

Figure 2. BT062 in a mouse xenograft breast cancer model

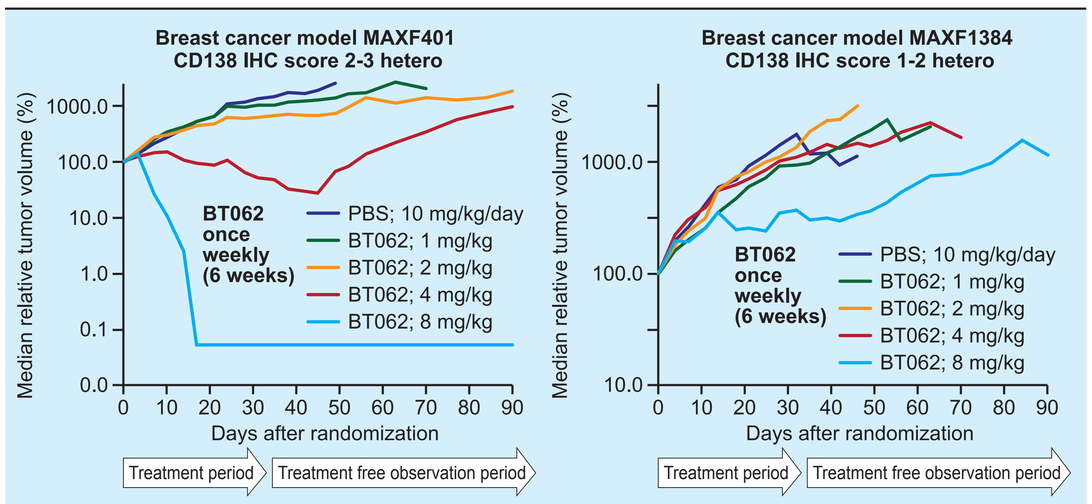


- BT062 treatment at 2 mg/kg or 4 mg/kg resulted in complete tumor remission (Figure 2b).
- Taxol administered on days 1, 8, 15, and 22 showed no anti-tumor effect, qualifying this model as a taxol-resistant animal model (Figure 2b).
- As a negative control, DM4 was administered separately and demonstrated no anti-tumor effect (Figure 2b).
- These data confirmed that efficacy of BT062 depends on targeted delivery of the maytansinoid DM4 to the antigen expressing tumor cells.
- All treatments were well tolerated as determined by monitoring for body weight changes.

Anti-tumor activity of BT062 is dependent on CD138 expression levels

- To characterize the correlation between tumor CD138 expression and efficacy of BT062, 2 different mammary carcinomas were xenografted onto NMRI nude mice.
- The MAXF401 model showed an IHC score for CD138 expression of 2-3 and the MAXF 1384 model showed an IHC score for CD138 expression of 1-2.
- BT062 treatment was at 1 of 4 doses (1 mg/kg, 2 mg/kg, 4 mg/kg, and 8 mg/kg) once weekly over a treatment period of 6 weeks.

Figure 3. Effect of BT062 on 2 breast cancer xenograft models



- In the MAXF401 model, treatment with 8 mg/kg resulted in complete tumor remission, but 4 mg/kg only resulted in a delay in tumor growth until after treatment was complete. Lower doses of BT062 had no significant effect (Figure 3).
- In the MAXF1384 model a tumor growth delay could be observed in the 8 mg/kg group. Lower doses had no significant effect in comparison to PBS treatment.

Summary

Table 3. Xenograft studies using primary patient-derived solid tumors expressing CD138

Tumor Indication	Treatment	Minimal Curative Dose	Time to complete response in all animals
Breast (triple negative)	6 weeks; once weekly	2 mg/kg	2.5 weeks
Bladder	5 weeks; once weekly	4 mg/kg	3 weeks
Lung	5 weeks; once weekly	4 mg/kg	4.5 weeks
Prostate	6 weeks; once weekly	8 mg/kg (2 mg/kg)*	4 weeks (5 weeks)
Pancreas	10 weeks; once weekly	13 mg/kg	7 weeks

* regrowth of tumors in 2/7 mice after end of treatment (6 weekly administrations).

- Data from *in vivo* evaluations of BT062 in solid tumors including the breast cancer data presented confirm an anti-tumor effect (Table 3).
- BT062 demonstrated dose-dependent antitumor activity in all models at doses with no significant toxicity as monitored by changes in mouse body weight.
- No antitumor activity was observed following administration of unconjugated BT062 antibody, or free DM4, demonstrating the importance of specific tumor-cell binding for BT062 to exert its cytotoxic activity.
- These results demonstrate the significant potential of BT062 for the treatment of solid tumors.
- There is a clear rationale to test the efficacy of BT062 in triple negative breast and bladder cancers. Phase I/II trials have been initiated in both indications. Further preclinical evaluations of the potential of BT062 in combination with chemotherapy are ongoing.

Disclosure
This work was funded by Biotest AG, the authors are all employees of Biotest.