

High in vivo anti-tumor activity of the immunoconjugate BT-062 against CD138 positive solid tumors

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Abstract # 226

CD138 (Syndecan-1) is highly upregulated on a variety of solid tumors and hematological malignancies and used as an identification marker for multiple myeloma (MM). Therefore, CD138 represents a promising target for therapeutic intervention in several malignancies. BT-062 is an immunoconjugate comprising a chimerized anti-CD138 antibody conjugated to maytansinoid (DM4), an inhibitor of tubulin polymerisation. We have previously reported that BT-062 exerts highly selective in-vitro and in-vivo cytotoxic activity against CD138 positive multiple myeloma (MM) cells [1]. Based on these results, a Phase I clinical trial has been conducted in relapsed/refractory multiple myeloma patients, which demonstrated overall good tolerability of BT-062 after repeated single dosing as well as first signs of efficacy in this heavily pretreated patient population [2]. Here, we further investigated the potential of BT-062 for the treatment of solid tumor indications. In accordance to published data, immunohistochemistry studies showed high CD138 expression in a variety of solid tumor indications, e.g. in pancreas, bladder, breast, head & neck, or lung carcinoma. Treatment of CD138 positive cell lines derived from these solid tumor indications exhibited selective cytotoxic activity down to subnanomolar IC₅₀ values. In order to assess BT-062's in-vivo efficacy, primary tumors (pancreatic, mammary, lung or transitional cell bladder carcinoma) were xenografted into SCID mice and treated by weekly intravenous injections. BT-062 demonstrated very high anti-tumor activity in all investigated primary xenograft models. Complete remission in all treated animals was observed in the pancreatic carcinoma model after 6 weeks treatment with 23.85 mg/kg or 13.25 mg/kg BT-062 (T/C = 0%) without tumor regrowth in any animal during treatment free observation period. Even when lower doses were tested (4 mg/kg), BT-062 treatment for 5 weeks resulted in complete tumor eradication (T/C = 0%) in all treated mammary carcinoma bearing animals without tumor regrowth in any animal. As the tumor displayed a triple negative phenotype (ER-, PR-, HER2-), BT-062 may become a future treatment option for this poor prognosis patient population. In conclusion - additionally to multiple myeloma - BT-062 exerts high in-vitro and in-vivo cytotoxic activity towards CD138 expressing solid tumors which may form the basis for clinical trials in these indications.

Background

CD138 / syndecan-1 is a transmembrane heparan sulfate proteoglycan exerting different functions such as mediation of cell proliferation, cell migration and cell-matrix interactions, contributing to the tumor cell interaction with surrounding tissues. BT-062 is an immunoconjugate directed against CD138 based on ImmunoGen's TAP (targeted antibody payload) technology consisting of approximately 3.5 DM4 molecules per antibody linker via a hindered disulfide linker. The conjugate is inactive in blood plasma and the original killing potency of the drug is restored only after internalisation into the CD138-expressing target cell.

Besides multiple myeloma, syndecan-1 overexpression has been detected in several solid tumor types such as in carcinomas of the pancreas, breast, prostate, colon, lung, endometrial ovarian, head and neck and bladder [3, 4]. Since BT-062 targets and kills CD138-positive multiple myeloma cells with high potency and specificity as previously demonstrated [1], preclinical studies were conducted to investigate the potential of BT-062 for the treatment of CD138 expressing solid tumors.

Methods

- CD138 expression was analysed on several tumorigenic cell lines by BT-062, using flow cytometry
- Patient-derived tumor tissue which had been directly transplanted from patients into mice (Oncotest GmbH, Freiburg, Germany) were screened for CD138 expression intensity and homogeneity by Immunohistochemistry (IHC). These primary human tumor xenotransplants retain most characteristics of the parental tumors including histology and response to standard anti-cancer drugs [5].
- Treatment of primary tumor xenografted mice with BT-062

Results

CD138 Expression and cytotoxic activity of BT-062 Against Different Cell lines

cell line	origin	Sensitivity IC ₅₀ (nM)	CD138 Expression receptors/cell
NCI-H929	MM	0.38 ⁽⁶⁾	788,752
PC-3	prostate cancer	0.79 ⁽⁴⁾	195,671
U266	MM	1.59 ⁽⁶⁾	782,987
MOLP-2	MM	1.78 ⁽⁶⁾	161,064
SK-BR-3	breast carcinoma	2.72 ⁽⁷⁾	444,350
LNCaP	prostate cancer	7.39 ⁽⁶⁾	23,388
CAPAN-2	pancreas carcinoma	15.51 ⁽²⁾	n. d.
PANC-1	pancreas carcinoma	36.38 ⁽⁶⁾	18,085
T47D	breast carcinoma	89.28 ⁽⁴⁾	42,264
Jurkat	T cell lymphoma	39.00 ⁽⁶⁾	0

⁽ⁿ⁾ mean of x experiments

Table 1. Selective cytotoxic activity of BT-062 was analysed against different tumor cell lines. CD138 expression (absolute number of CD138 receptors) has been analysed quantitatively in parallel by an indirect immunofluorescence assay (Quikfit[®], Dako) using the unconjugated antibody nT062. Sensitivities towards BT-062 (determined as cytotoxicity) of the cells are given as IC₅₀ values (average values of independent measurements).

CD138 positive tumors are completely eradicated by the anti-CD138 Immunoconjugate BT-062 in mice xenografts

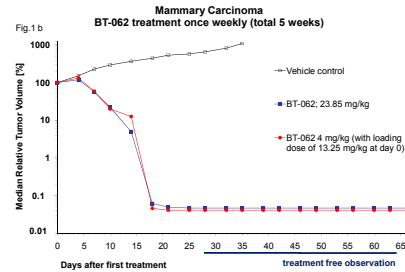
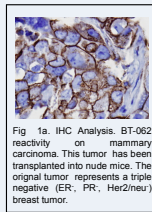


Fig. 1b NMR1 nu/nu mice were subcutaneously implanted with the mammary tumor (Fig. 1a). Treatment with BT-062 at two dose levels was initiated when tumors reached a size of approx. 115 mm³ (Day 0). Mice were treated intravenously once weekly. Tumors were completely eradicated 18 days after the first treatment (T/C = 0). No tumor re-growth was observed in a 39 days treatment free observation period.

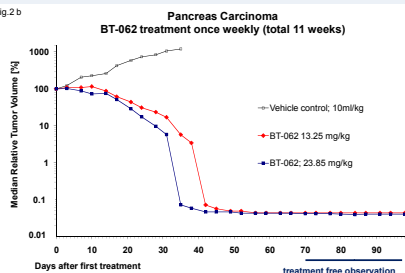
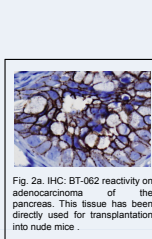


Fig. 2b NMR1 nu/nu mice were subcutaneously implanted with the pancreas tumor shown in Fig. 2a. Treatment with BT-062 at two dose levels was initiated when tumors reached a size of approx. 115 mm³ (Day 0). Mice were treated intravenously once weekly with the conjugate (total 11 weeks). Complete remission was observed 35 days after the first treatment (T/C = 0). No re-growth was observed in a treatment free period of 28 days confirming that the tumors were completely eradicated.

BT-062 treatment leads to complete eradication of CD138 positive bladder and lung tumors

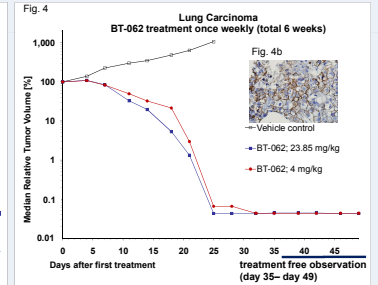
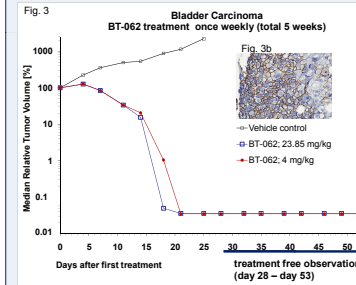


Fig. 3 NMR1 nu/nu mice were subcutaneously implanted with a patient derived bladder tumor (Fig. 3b). When tumors reached a size of approx. 150 mm³, treatment with BT-062 once weekly at two doses was initiated. (25 days after the first treatment, tumors were completely eradicated (T/C = 0%). After 5 weeks of BT-062 injection and a treatment free period of 25 days, no tumor regrowth was observed.

Fig. 4 NMR1 nu/nu mice were subcutaneously implanted with a patient derived lung tumor (primary adenocarcinoma; Fig. 4b). When tumors reached a size of approx. 115.2 mm³, treatment with BT-062 once weekly at two doses was initiated. (21 days after the first treatment, tumors were completely eradicated (T/C = 0%). After 6 injections with BT-062 and a treatment-free period of 14 days, no tumor regrowth was observed.

Summary and Conclusion

- High reactivity of BT-062 was observed by IHC on patient derived tumors

- Xenograft studies of patient derived tumors:
 - Eradication of all investigated CD138 positive tumors by BT-062 with any dose evaluated
 - lowest dose tested equivalent to clinical applied dose, i.e. below the maximum tolerated dose
 - Robust tumor response (T/C = 0% after 3-6 BT-062 injections)
 - No tumor re-growth

Tumor	Histology	Day of complete response (T/C = 0%)	Re-growth	Treatment
Mammary	adenocarcinoma	18	No	once weekly (5 weeks)
Pancreas	adenocarcinoma	35	No	once weekly (11 weeks)
Bladder	urothelial carcinoma	21	No	once weekly (5 weeks)
Lung	adenocarcinoma	25	No	once weekly (5 weeks)

Table 2. Summary of all conducted xenograft studies. T/C: Tumor growth inhibition (median tumor volume treated / median tumor volume control x 100)

→ BT-062 represents a potential therapeutic agent for the treatment of CD138 positive solid tumors

References

- [1] Ikeda et al., 2009, Clin Cancer Res, 15(12): 4028-4037.
- [2] Chanan-Khan et al., ASH 2009. Poster
- [3] Saqi et al., 2005, Diagn Cytopathol., 33(2): 65-70.
- [4] Anttonen et al., 1999, Br J Cancer, 79(3-4):558-64.
- [5] Fiebig et al., 2001, Tumor models in Cancer Research. In Teicher BA: 113-137.