The identification of the AXL/Gas6 signalling axis as a key player of myelodysplastic syndrome (MDS) and the potential of the oral selective AXL inhibitor bemcentinib in the treatment of MDS

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Background & Study Objectives

Myelodysplastic syndrome (MDS)

Myelodysplastic syndrome (MDS) is a stem cell driven disorder that is characterised by inefficient haematopoiesis and a high risk of progression to acute myeloid leukaemia. MDS stem cells are defined as lin-CD34+CD38- (MDS-SCs)[1,2]. We have previously shown that there is a strong dependency of MDS-SCs on disease-associated mesenchymal niche cells (MDS-MSCs)[2]. Compared to healthy cells, MDS-MSCs display de-regulation of several niche factors involved in intercellular communication, including GAS6, a high affinity AXL ligan

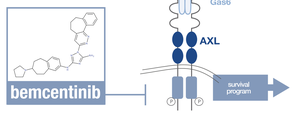
Study objectives

In this study we explored the functional importance of GAS6/AXL axis in MDS pathogenesis using both in vitro and in vivo assays. We used bemcentinib (BGB324), a selective orally bioavailable small molecule inhibitor of AXL in phase II clinical development, to demonstrate the potential therapeutic benefit of targeting GAS6/AXL axis in human MDS.

Bemcentinib, first-in-class, highly selective orally bioavailable AXL inhibitor in phase II

Bemcentinib selectivity profile

	Kinome Scan (Kd)		Kinase Profiler (IC50)		Fluorescence Polarization (IC_{50})		Receptor Cross-Linking (IC_{50})	
Kinase	nM	Fold	nM	Fold	nM	Fold	nM	Fold
Axl	0.4	1	4.6	1	14	1	14	1
Mer	100	250	12.5	2.7	220	16		50
Tyro	>1000	>1000	413	89				>100



Background

Bemcentinib (BGB324) is a first-in-class, oral selective inhibitor of the RTK AXL currently in Ph II clinical development across several cancer types. AXL overexpression has been established as an independent negative prognostic factor in AML whereas AXL inhibition via bemcentinib has shown anti- leukemic activity and immune activation in pre-clinical models of AML and other cancers.

Bemcentinib clinical development

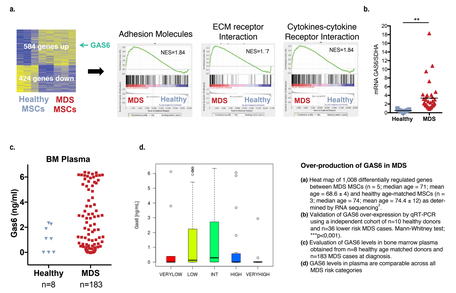
Bemcentinib is being explored as a mono-therapy and in combination with immune-, targeted and chemo-therapy in AML/MDS, NSCLC, TNBC and melanoma across six phase II clinical trials.

Methods

GAS6 levels in bone marrow plasma were evaluated using the DY885B human GAS6 DuoSet ELISA from R&D. FFPE material from bone marrow trephine biopsies were used to generate 3µm section slides and stained using a mouse monoclonal anti-human AXL antibody at 1ug/ml (clone 1H12)[3] using a citrate buffer antigen retrieval protocol. Staining was performed with the Leica Bond-Max using the Bond Polymer Refine Detection Kit (Leica).
In vitro 2D-coculture of MDS cells on MSCs were used to evaluate the impact of bemcentinib on MDS growth ex vivo. Colony forming assays were performed using a complete cytokine containing Methocult medium (#H4434, Stem Cell Technologies). Ex-vivo treatment with bemcentinib was done at a maximal dose of 1 uM. For in vivo experiments, bemcentinib was administered at 50mg/kg by oral gavage twice daily.

Results

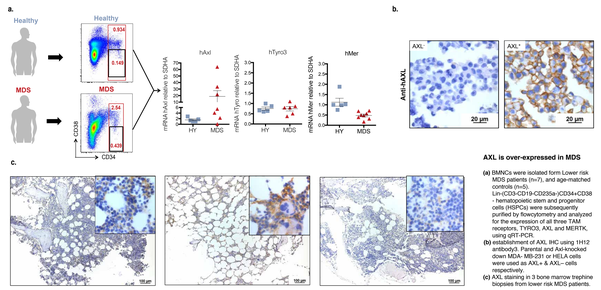
GAS6 is upregulated in MDS-derived mesenchymal niche cells



Over-production of GAS6 in MDS

(a) Heat map of 1,008 differentially regulated genes between MDS MSCs (n = 5; median age = 71; mean age = 68.6 ± 4) and healthy age-matched MSCs (n = 3; median age = 74; mean age = 74.4 ± 12) as determined by RNA sequencing.
(b) Validation of GAS6 over-expression by qRT-PCR using a independent cohort of n=10 healthy donors and n=36 lower risk MDS cases. Mann-Whitney test; **p<0.001).
(c) Evaluation of GAS6 levels in bone marrow plasma obtained from n=8 healthy age matched donors and n=183 MDS cases at diagnosis.
(d) GAS6 levels in plasma are comparable across all MDS risk categories

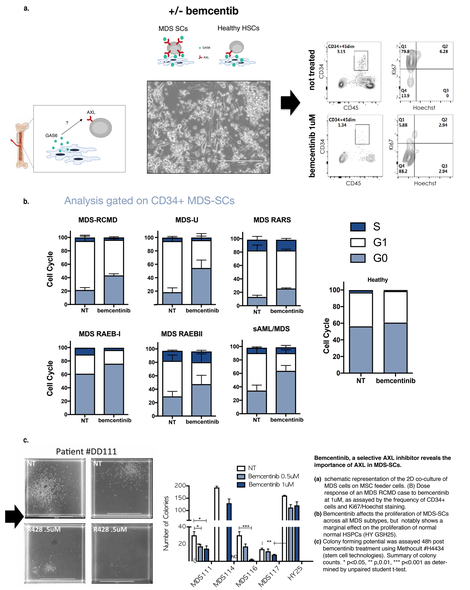
AXL is readily detectable in bone marrow trephine biopsies from lower-risk MDS & is the only TAMR over-expressed in MDS stem/progenitor cells



AXL is over-expressed in MDS

(a) BMNCs were isolated form Lower risk MDS patients (n=7), and age-matched controls (n=5).
Lin-/CD3-/CD19-/CD235a-/+/CD34+/CD38 - hematopoietic stem and progenitor cells (HSPCs) were subsequently purified by flowcytometry and analyzed for the expression of all three TAM receptors, TYRO3, AXL and MERTK, using qRT-PCR.
(b) establishment of AXL IHC using 1H12 antibody5. Parental and Axl-knocked down MDA- MB-231 or HELA cells were used as AXL+ & AXL- cells respectively.
(c) AXL staining in 3 bone marrow trephine biopsies from lower risk MDS patients.

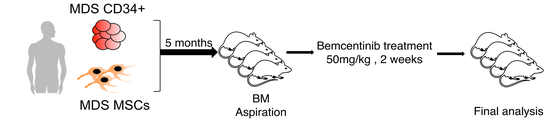
Bemcentinib reduces the frequency, the proliferation and the clonogenic potential of CD34+ MDS stem /progenitor cells ex-vivo

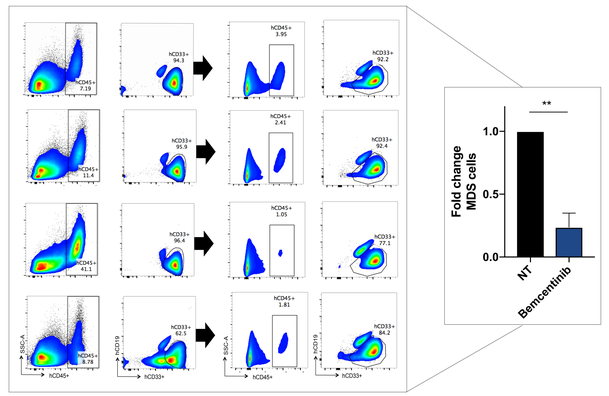


Bemcentinib, a selective AXL inhibitor reveals the importance of AXL in MDS-SCs.

(a) schematic representation of the 2D co-culture of MDS cells on MSC feeder cells. (B) Dose response of an MDS RCMD case to bemcentinib at 1uM, as assayed by the frequency of CD34+ cells and Ki67/Hoechst staining.
(b) Bemcentinib affects the proliferation of MDS-SCs across all MDS subtypes, but notably shows a marginal effect on the proliferation of normal normal HSPCs (HY GSH25).
(c) Colony forming potential was assayed 48h post bemcentinib treatment using Methocult #H4434 (stem cell technologies). Summary of colony counts. * p<0.05, ** p 0.01, *** p<0.001 as determined by unpaired student t-test.

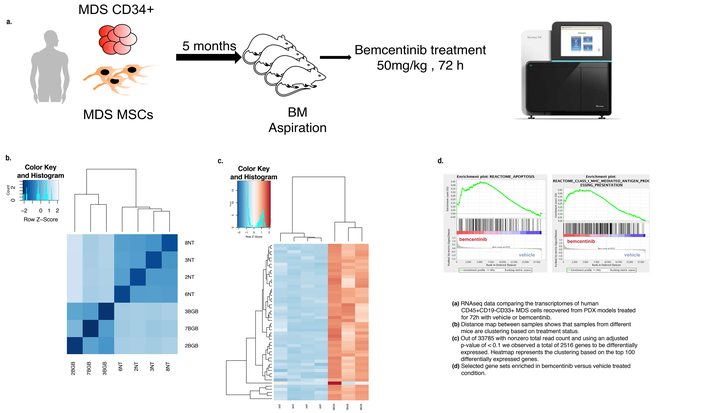
Bemcentinib reduces MDS burden in a PDX model of MDS





Human CD45, CD19 and CD33 evaluation in PDX of lower risk MDS before and after Bemcentinib treatment at a dose of 50mg/kg.

Molecular effects of bemcentinib on MDS cells in vivo



(a) RNAseq data comparing the transcriptomes of human CD45+/CD19-/CD33+ MDS cells recovered from PDX models treated for 72h with vehicle or bemcentinib.
(b) Distance map between samples shows that samples from different mice are clustering based on treatment status.
(c) Out of 33785 with nonzero total read count and using an adjusted p-value of < 0.1 we observed a total of 2516 genes to be differentially expressed. Heatmap represents the clustering based on the top 100 differentially expressed genes.
(d) Selected gene sets enriched in bemcentinib versus vehicle treated condition.

Conclusions

- Gas6 is over-expressed in mesenchymal niche cells from lower-risk MDS patients.
- Comparable Gas6 levels were observed across all MDS risk categories.
- AXL, the high affinity GAS6 receptor is the only TAM receptor up-regulated in purified CD34+ MDS stem/progenitor cells.
- AXL protein expression is readily detectable by IHC in bone marrow trephine biopsies from lower-risk MDS patients.
- Ex-vivo treatment with bemcentinib, reduces the frequency, the proliferation and the clonogenic potential of CD34+ MDS-SCs. This effect is reproducible across all MDS risk categories and only marginaly seen in CD34+ cells derived from age-matched controls.
- In vivo, Bemcentinib inhibited MDS propagation as determined by a decrease in the frequency of human CD45+CD33+ MDS cells in a PDX model of MDS.
- Bemcentinib is a selective, orally bioavailable small molecule inhibitor of AXL currently in phase II clinical development in AML/MDS, NSCLC, TNBC and melanoma.

References and Funding

References
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