INHIBITION OF S100A9 WITH TASQUINIMOD DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN PRE-CLINICAL MODELS OF MULTIPLE MYELOMA

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INTRODUCTION

Multiple myeloma (MM) is an incurable hematological malignancy characterized by clonal proliferation of plasma cells localized preferentially in the bone marrow (BM). Despite the emergence of novel therapeutics, MM remains a fatal disease. The tumor microenvironment plays a critical role in promoting MM growth and we have recently demonstrated that a population of myeloid-derived suppressor cells in the BM microenvironment is involved in regulation of MM progression. These cells abundantly produce the protein S100A9 which has recently been implicated in the development of cancer. Tasquinimod (ABR-215050, Active Biotech, Lund, Sweden) is an investigational drug that binds the S100A9 protein and inhibits the interactions with its receptors.

OBJECTIVE

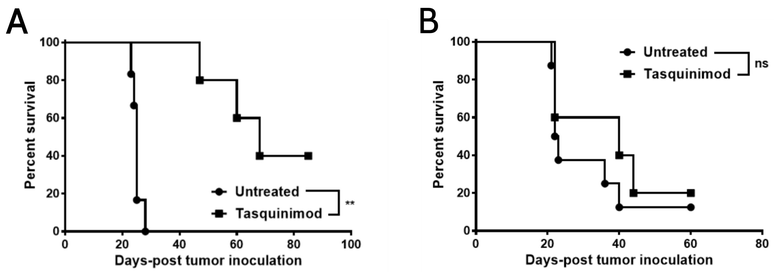
To investigate the anti-tumor effects of tasquinimod as a single agent and in combination with standard therapeutics in pre-clinical models of MM.

METHODS

Syngeneic and xenograft mouse models of MM were used to evaluate anti-tumor effect of tasquinimod. In a syngeneic model, DP42 cells were injected intravenously into wild type and S100A9 knockout (KO) mice. Tasquinimod was administered via drinking water beginning the day after tumor inoculation and survival was determined when mice reached the humane endpoint. For xenograft models, MM tumors were established by subcutaneous injection of H929, RPMI-8226 or MM1S human MM cells into NSG mice. Mice were treated with tasquinimod (30mg/kg/day) and lenalidomide (oral gavage, 5mg/kg/day), bortezomib (every 4 days, i.v., 0.5mg/kg), or dexamethasone (i.p., 10mg/kg) once tumors were measurable (approx. day 14 – 20 after inoculation). Immunohistochemical staining of the bone marrow was performed using anti-CD31 antibodies. Serum levels of angiogenic factors was determined using a Mouse Angiogenesis Proteome Profiler Antibody Array (R&D). The *in vitro* effect of tasquinimod on the viability and apoptosis of MM cells was evaluated by MTT assay and by flow cytometry using Annexin V/DAPI staining.

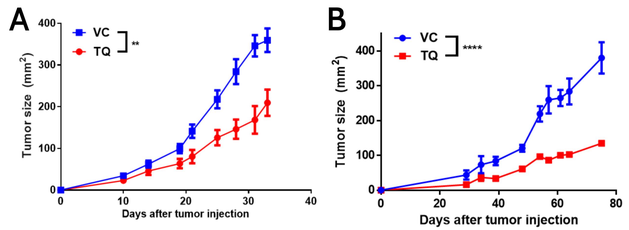
RESULTS

Tasquinimod demonstrated a potent anti-MM effect in a syngeneic model of MM



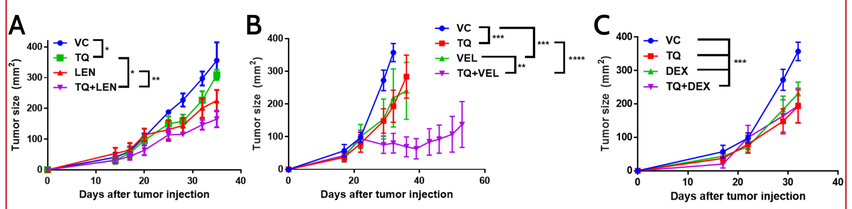
In a syngeneic model, **(A)** tasquinimod significantly prolonged the survival of MM-bearing mice compared to the vehicle-treated group (p<0.005). However, tasquinimod did not improve the survival of **(B)** MM-bearing S100A9KO mice, indicating that the observed anti-MM effect is indeed mediated through the inhibition of S100A9.

Tasquinimod demonstrated potent anti-MM effects in xenograft models of MM



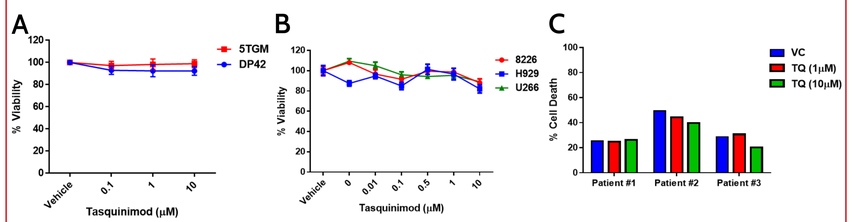
In xenograft models of human MM, tasquinimod (TQ) used as a single agent significantly reduced the growth of human **(A)** H929 (p=0.0042) and **(B)** RPMI-8226 (p<0.0001) tumors established in NSG mice compared to tumors in vehicle (VC) treated mice.

Tasquinimod demonstrated strong anti-MM effects in combination with existing therapies



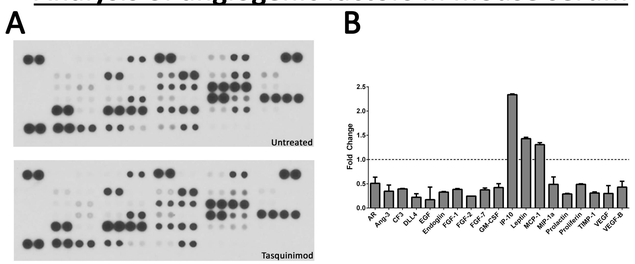
In xenograft models of MM, the combinations of **(A)** tasquinimod and lenalidomide (TQ+LEN) significantly reduced the growth of MM1.S tumors (p<0.01) and **(B)** tasquinimod and bortezomib (TQ+VEL) drastically decreased the growth of H929 tumors in NSG mice (p<0.0001) compared to treatments with the single agents alone. However, **(C)** the combination of tasquinimod and dexamethasone (TQ+DEX) did not improve the anti-MM effects of the single agents.

Tasquinimod does not affect MM cell viability or apoptosis in vitro



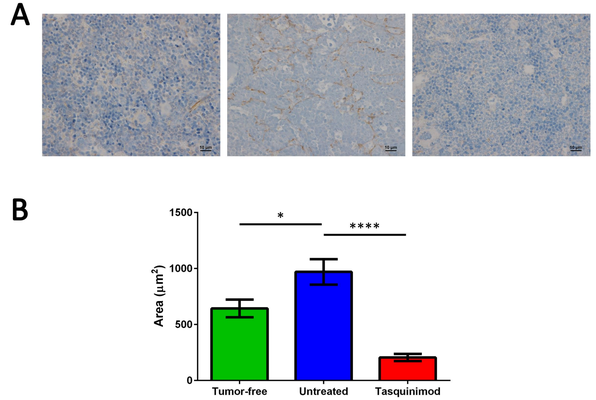
Treatment with Tasquinimod for 24hrs did not affect the viability of **(A)** murine MM cell lines or **(B)** human MM cell lines *in vitro*. **(C)** Tasquinimod treatment of CD138+ cells from patients also did not induce apoptosis over 24hrs. This suggests that the *in vivo* anti-MM effect of tasquinimod was mediated by alterations of the tumor microenvironment.

Analysis of angiogenic factors in mouse serum



(A) Mouse Angiogenesis Proteome Profiler Antibody Array (R&D) analyzing serum from untreated mice *(top)* and mice treated with tasquinimod *(bottom)*. **(B)** Graph of fold change in angiogenic factors following tasquinimod treatment. A significant decrease in VEGF, FGF2, tissue factor, and endoglin was detected in tasquinimod-treated mice.

Tasquinimod decreased BM angiogenesis in MM-bearing mice



(A) Immunohistochemical analysis of CD31 expression in the BM of tumor-free *(left)*, MM-bearing *(middle)* and tasquinimod-treated MM-bearing *(right)* mice. **(B)** Quantification of CD31 staining in femoral sections of 3 mice from each group. Increased angiogenesis in the BM of MM-bearing mice was observed as compared with control tumor-free mice (* p=0.0231). Treatment with tasquinimod significantly reduced angiogenesis (**** p<0.0001).

SUMMARY

Our data demonstrates that targeting S100A9 with tasquinimod results in a strong anti-tumor effect in syngeneic and xenograft models of MM, both as monotherapy and in combination with standard anti-MM therapies. There was no effect on the viability and apoptosis of cells in vitro suggesting that anti-MM effects are mediated by alterations to the tumor microenvironment. We also show that the anti-MM effect of tasquinimod was associated with reduced angiogenesis in the BM. The pronounced anti-tumor effects of tasquinimod in combination with standard anti- MM therapies (proteasome inhibitors (bortezomib) and the immunomodulating agent (lenalidomide)) suggests that different tasquinimod combination strategies represent a novel and promising treatment in multiple myeloma.