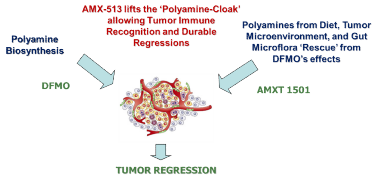
AMX-513 polyamine depletion therapy inhibits tumor growth and reverses immunosuppression in cancers including MYC-driven neuroblastoma and pancreatic cancer

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Introduction

- Recent data supports an immunosuppressive role of tumor polyamines.
- Increased concentrations of polyamines in the tumor microenvironment are associated with Ras and Myc-induced increased biosynthesis via ornithine decarboxylase (ODC).
- AMX-513 combines polyamine ***Biosynthesis*** (DFMO) and ***Transport*** (AMXT 1501) inhibition and highlights immune-mediated tumor regressions and MDSC reduction in tumor microenvironment.



AMX-513 *in vitro* pharmacology

- AMXT 1501 is a nanomolar potency inhibitor (10.5 nM) of the extracellular transporter of polyamines.
- AMX-513 combines polyamine ***Biosynthesis*** (DFMO) and ***Transport*** (AMXT 1501) inhibitors and significantly decreases cellular and tumor polyamine levels.
- Cytostatic growth inhibition against multiple tumor lines.

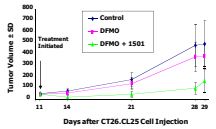
Cellular AMXT 1501 data as polyamine transport inhibitor	
1	***Ability*** to inhibit the uptake of radiolabeled spermidine into MDA-MB-231 breast cancer cells (K_i value = 10.5 nM for ^{3}H-spd uptake).
2	***Ability*** to increase the growth inhibitory effects of DFMO on cells in culture even in the presence of 1.0 μM extracellular spermidine.
3	***Inability*** to rescue cells from the growth inhibitory effects of DFMO in the absence of extracellular polyamines.
4	***Ability*** to deplete the intracellular levels of polyamines after combination treatment with DFMO.

AMX-513 efficacy requires intact T cell population

Balb/c mice:

Mice with immune system

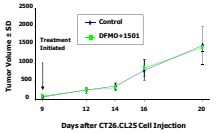




Athymic nude mice:

Mice lacking T cells



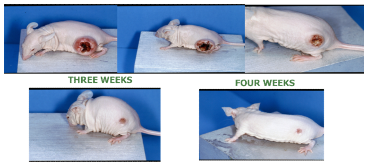


Murine CT26.CL25 syngeneic colorectal cancer. (n = 5 per treatment group): VC, DFMO (400 mg/kg oral, daily), AMXT 1501 (3.0 mg/kg IP BID daily).

In vivo efficacy of AMX-513

A. Skin cancer (K6/ODC) murine model

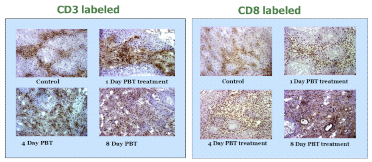
Chemically-induced tumors in transgenic immunocompenent K6/ODC mice



Immune-competent mice (K6/ODC) treated with PaBT: AMXT 1501 i.p. twice daily (3.0 mg/kg) combined with 0.5% DFMO in drinking H_2O

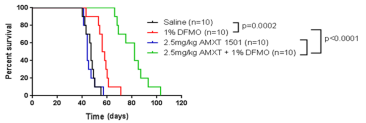
O'Brien, *Int J Cancer* **2006**
Burns, *J Med Chem* **2009**

Cytolytic Tumor Infiltrating T Lymphocytes (CTL) induced by AMX-513 treatment



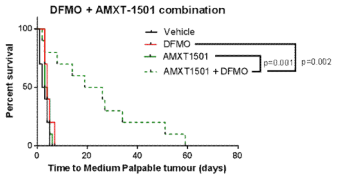
Conditions: Mouse tumor sections from K6/ODC animals analyzed by immunohistochemistry after AMX-513 treatment at the times shown.

B. TH-MYCN Prophylaxis with AMXT 1501

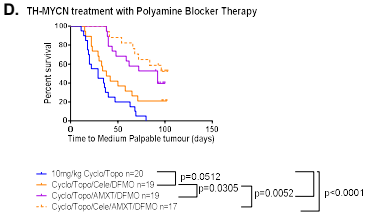


Prophylactic treatment of TH-MYCN mice initiated when mice pups were weaned. AMX-513 consists of AMXT 1501 4HCl 2.5 mg/kg SC + 1% DFMO in drinking H_2O until time to medium palpable tumor.

C. TH-MYCN treatment with AMX-513

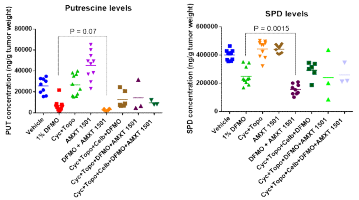


D. TH-MYCN treatment with Polyamine Blocker Therapy



Therapeutic treatment of TH-MYCN mice (C and D) initiated in 3 week old mice when tumors were ~5mm in size; cyclophosphamide, 20mg/kg, QDx5, IP; topotecan, 0.5mg/kg, QDx5, IP; Celebrex, 100 mg/kg, 5 days on, 2 days off, for 6 weeks; AMXT 1501 2.5 mg/kg/day QDx42, SC.; DFMO, 1% in drinking water

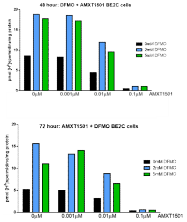
AMX-513-Induced Polyamine Reduction in Transgenic TH-MYCN Neuroblastoma Mice



Conditions: Putrescine (PUT) and spermidine (SPD) levels in tumors from TH-MYCN mice on Day 66. Treatment: cyclophosphamide, 20mg/kg, QDx5, IP; topotecan, 0.5mg/kg, QDx5, IP; Celebrex, 100 mg/kg, 5 days on, 2 days off, for 6 weeks; AMXT 1501 2.5 mg/kg/day QDx42, SC; DFMO, 1% in drinking water.

AMX-513 Inhibits uptake of Polyamine into Neuroblastoma cells

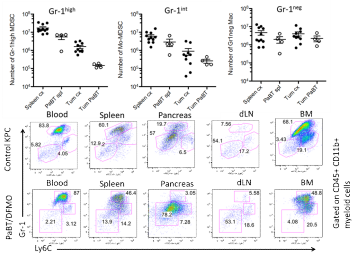
Uptake of Radiolabelled spermidine (48 and 72 hours, BE2C cells)



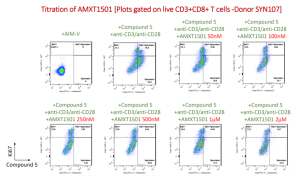
MDSC Reduction in AMX-513 Treated KPC Mice

Pilot study in KPC transgenic mouse pancreatic tumors

- **5 animals in study** (AMXT 1501 s.c. QID 2.5 mg/kg and 0.5% DFMO in drinking water)
- **No drug associated adverse effects observed** (4 mice treated for 28 days, 1 mouse for 14 days)
- **1 animal died from progressive disease, 2 animals had no progression**
- **2 animals had full tumor regression** (no tumor visible by ultrasound)



Ex Vivo Pharmacodynamic (PD) Assay in Whole Human Blood is Potently inhibited by 100 nM AMXT 1501



Clinical Development Strategy for AMX-513

- **Phase 1a – AMXT 1501 alone in Normal Healthy Volunteers (June, 2017)**
  - Safety
  - AMXT 1501 PK plasma levels after oral dosing
  - PD target engagement of polyamine uptake into whole blood cells
- **Phase 1b – AMXT 1501 plus ascending DFMO (December, 2017)**
  - Safety of AMXT 1501 in combination with DFMO
  - DFMO PK and PD biomarker
  - Polyamine and immune target engagement

AMX-513 Summary

Polyamine Depletion by AMXT 1501 (Transport) and DFMO (Biosynthesis) Inhibitor Combination

- **Potent cytostatic cancer cell growth inhibition in vitro**
- **Tumor regression in multiple MYC and RAS driven models in vivo**
- **Tumor cell intrinsic (polyamine anti-metabolite) and TME extrinsic (immune) mechanistic components.**

Bibliography

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2. Hayes, C.S.; Shicora, A.C.; Keough, M.P., Snook, A.E.; Burns, M.R., Gilmour, S.K. (2014) *Cancer Immunol. Res.*, 2(3), 274-85.
3. Samal, K., Shao, P., Kendzicky, A., Yco, L.P., McClung, H., Gerner, E., Burns, M., Bachmann, A.S., Sholler, G. (2013), *Int. J. Cancer.* 133(6), 1323-33.

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